Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Issue Date: 12/05/2007

Grant Number: 1R01AI070908-01A2

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Asst. Vice Provost for Research
PreAward Services
2 Fairchld Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Budget Period: 12/15/2007 – 11/30/2008
Project Period: 12/15/2007 – 11/30/2012

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $365,000 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Grant Number R01AI070908 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health."

Award recipients are strongly encouraged to submit to PubMed Central (PMC), upon acceptance for publication, an electronic version of peer-reviewed, original research publications, resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Mollie Shea
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Obtained via FOIA by White Coat Waste Project

SECTION I – AWARD DATA – 1R01AI070908-01A2

Award Calculation (U.S. Dollars)

Federal Direct Costs	$250,000
Federal F&A Costs	$115,000
Approved Budget	$365,000
Federal Share	$365,000
TOTAL FEDERAL AWARD AMOUNT	$365,000

AMOUNT OF THIS ACTION (FEDERAL SHARE) $365,000

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$365,000	$365,000
2	$365,000	$365,000
3	$365,000	$365,000
4	$365,000	$365,000
5	$365,000	$365,000

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908A
Fiscal Year:	2008

IC	*CAN*	*2008*	*2009*	*2010*	*2011*	*2012*
AI	8472350	$365,000	$365,000	$365,000	$365,000	$365,000

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C / **OC**: 414A / **Processed**: eRA Commons User Name 11/29/2007

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AI070908-01A2

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01AI070908-01A2

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. The restrictions on the expenditure of federal funds in appropriations acts to the extent those restrictions are pertinent to the award.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

Obtained via FOIA by White Coat Waste Project

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1R01AI070908-01A2

To meet Institute, program objectives, this grant is funded at the IRG/Council recommended level.

This is a Modular Award without direct cost categorical breakdowns in accordance with the guidelines published in the NIH Grants Policy Statement, December 2003, see (http://grants2.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600227), pages 217-219. Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Mollie Shea
Email: mshea@niaid.nih.gov **Phone**: 301-402-6576 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sp189u@nih.gov **Phone**: 301-496-7728 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AI070908-01A2

INSTITUTION: KANSAS STATE UNIVERSITY

Budget	*Year 1*	*Year 2*	*Year 3*	*Year 4*	*Year 5*
TOTAL FEDERAL DC	$250,000	$250,000	$250,000	$250,000	$250,000
TOTAL FEDERAL F&A	$115,000	$115,000	$115,000	$115,000	$115,000
TOTAL COST	$365,000	$365,000	$365,000	$365,000	$365,000

Facilities and Administrative Costs	*Year 1*	*Year 2*	*Year 3*	*Year 4*	*Year 5*
F&A Cost Rate 1	46%	46%	46%	46%	46%
F&A Cost Base 1	$250,000	$250,000	$250,000	$250,000	$250,000
F&A Costs 1	$115,000	$115,000	$115,000	$115,000	$115,000

Obtained via FOIA by White Coat Waste Project

PI: **GANTA, ROMAN R.**	Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression	
Received: 03/19/2007	FOA: PA07-070	Council: 10/2007
Competition ID:	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
1 R01 AI070908-01A2	Dual:	Accession Number: 2982281
IPF: 4202801	Organization: KANSAS STATE UNIVERSITY	
Former Number:	Department: Diagnostic Medicine/Pathobiolo	
IRG/SRG: ZRG1 IDM-B (02)M	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 250,000 Year 2: 250,000 Year 3: 250,000 Year 4: 250,000 Year 5: 250,000	Animals: N Humans: N Clinical Trial: N Current HS Code Evaluative Info HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Roman Ganta	Kansas State University	PD/PI
Redacted by agreement		Other (Specify)-Collaborator
		Other (Specify)-Collaborator

Appendices

Ganta-Appendix 4,Appendix Material,Ganta-Appendix 3

Obtained via FOIA by White Coat Waste Project

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
03/19/2006	
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

❍ Pre-application ● Application
❍ Changed/Corrected Application

4. Federal Identifier
1R01 AI070908-01A1

5. APPLICANT INFORMATION *** Organizational DUNS:**929773554

* Legal Name: Kansas State University
Department:
Division:
* Street1: 2 Fairchild Hall
Street2:
* City: Manhattan
County: Riley
* State: KS: Kansas
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 66506-1103

Person to be contacted on matters involving this application

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Mr.	Paul	R.	Lowe	

* Phone Number: 785-532-6804
Fax Number: 785-532-5944
Email: plowe@ksu.edu

6. * EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)*:
1480771751A1

7. * TYPE OF APPLICANT
H: Public/State Controlled Institution of Higher Education

8. * TYPE OF APPLICATION: ❍ New
● Resubmission ❍ Renewal ❍ Continuation ❍ Revision

Other (Specify):

Small Business Organization Type
❍ Women Owned ❍ Socially and Economically Disadvantaged

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)*:

9. * NAME OF FEDERAL AGENCY:
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

* Is this application being submitted to other agencies? ❍ Yes ● No
What other Agencies?

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*
All

13. PROPOSED PROJECT:

* Start Date	* Ending Date
12/01/2007	11/30/2012

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant	b. * Project
02	KS-002

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Dr.	Roman	Reddy	Ganta	

Position/Title: Professor
* Organization Name: Kansas State University
Department: Diagnostic Medicine/Pathobiolo
Division: College of Veterinary Medicine
* Street1: 1800 Denison Avenue
Street2:
* City: Manhattan
County: Riley
* State: KS: Kansas
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 66506
* Phone Number: 785-532-4612
Fax Number: 785-532-4039
* Email: rganta@vet.ksu.edu

Obtained via FOIA by White Coat Waste Project

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding	$1,825,000.00
b. * Total Federal & Non-Federal Funds	$1,825,000.00
c. * Estimated Program Income	$0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● * I agree

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

19. Authorized Representative

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Mr.	Paul	R.	Lowe	

* Position/Title: Asst. Vice Provost for Research
* Organization Name: Kansas State University
Department: PreAward Services
Division:
* Street1: 2 Fairchild Hall
Street2:
* City: Manhattan
County: Riley
* State: KS: Kansas
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 665061103
* Phone Number: 785-532-6804
Fax Number: 785-532-5944
* Email: plowe@ksu.edu

*** Signature of Authorized Representative**

Paul Lowe

*** Date Signed**

03/19/2007

20. Pre-application File Name: Mime Type:

21. Attach an additional list of Project Congressional Districts if needed.

File Name: Mime Type:

Obtained via FOIA by White Coat Waste Project

424 R&R and PHS-398 Specific Table Of Contents

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Appendix

Number of Attachments in Appendix: 3

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: Kansas State University

* Street1: 1600 Denison Avenue		Street2:
* City: Manhattan	County: Riley	* State: KS: Kansas
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 66506

File Name	Mime Type

Additional Location(s)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved?	❍ Yes	● No
1.a. If YES to Human Subjects		
Is the IRB review Pending?	❍ Yes	❍ No
IRB Approval Date:		
Exemption Number:	_ 1 _ 2 _ 3 _ 4 _ 5 _ 6	
Human Subject Assurance Number		
2. * Are Vertebrate Animals Used?	❍ Yes	● No
2.a. If YES to Vertebrate Animals		
Is the IACUC review Pending?	❍ Yes	❍ No
IACUC Approval Date:		
Animal Welfare Assurance Number		
3. * Is proprietary/privileged information included in the application?	❍ Yes	● No
4.a. * Does this project have an actual or potential impact on the environment?	❍ Yes	● No
4.b. If yes, please explain:		
4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed?	❍ Yes	❍ No
4.d. If yes, please explain:		
5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators?	❍ Yes	● No
5.b. If yes, identify countries:		
5.c. Optional Explanation:		

6. * Project Summary/Abstract	1945-Ganta-Abstract.pdf	Mime Type: application/pdf
7. * Project Narrative	297-Ganta-Project_narrative.pdf	Mime Type: application/pdf
8. Bibliography & References Cited	2491-Ganta-references.pdf	Mime Type: application/pdf
9. Facilities & Other Resources	8746-Ganta-Facilitie.pdf	Mime Type: application/pdf
10. Equipment		

Obtained via FOIA by White Coat Waste Project

PROJECT ABSTRACT:

Illnesses caused by the tick-borne rickettsiales of the genera *Ehrlichia* and *Anaplasma* have been a growing health concern in recent years. These illnesses include human monocytic ehrlichiosis, human granulocytic ehrlichiosis and human granulocytic anaplasmosis which are caused by *Ehrlichia chaffeensis, E. ewingii* and *Anaplasma phagocytophilum*, respectively. Despite the complex cellular environment of arthropods and vertebrates having sophisticated systems of defense, the rickettsiales evolved strategies to evade host clearance. For example, *E. chaffeensis* in macrophages and tick cells differ significantly in their morphology with distinct patterns of protein expression. The expression patterns include the differential expression from the p28-Omp multigene locus which contains 22 genes. The p28 Omp gene expression is restricted primarily to the p28-Omp gene 19 in macrophages, whereas in tick cells the expressed antigen is predominantly from the p28-Omp gene 14. Our studies also demonstrate that the differential expression in vertebrate and tick cells contributes to the altered host response, such as the delayed clearance in a vertebrate host for tick cell-derived *E. chaffeensis* compared to that for macrophage-derived bacteria. This study will focus on the basic understanding of the regulation of gene expression from the p28-Omp multigene locus. The central hypothesis of this study is that the transcription from the p28-Omp locus in *E. chaffeensis* is differentially regulated in response to environmental signals and that the host cell-specific gene expression is essential for pathogen's survival in vertebrate and tick cell environments. Specific aims of this study are; 1) establish in vitro transcription and translation system for mapping *Ehrlichia* promoters, 2) map transcriptional machinery in differentially expressed genes 14 and 19 of the p28-Omp locus, 3) evaluate the contributions of macrophage and tick cell environments for differential expression from genes 14 and 19, and 4) evaluate knockout mutations for the p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific expression. The results from this study will provide important information for understanding *E. chaffeensis* gene regulation and how the rickettsiale in macrophages and tick cells respond to the loss of expression from a differentially expressed protein.

Obtained via FOIA by White Coat Waste Project

Project narrative:

The results from this study will provide important information for understanding *Ehrlichia chaffeensis* pathogenesis, gene regulation and how the rickettsiale in macrophages and tick cells respond to the loss of expression from a differentially expressed protein. This study will ultimately allow us to determine how the tick-transmitted pathogen and other closely related pathogens persist in a vertebrate host and ultimately aids in the identification of targets for controlling infections.

Obtained via FOIA by White Coat Waste Project

Facilities and Research Environment of Dr. Roman Reddy Ganta

Laboratories: Dr. Ganta has three well-equipped research laboratories (450 sq. ft. each). Two laboratories have been designated for use in studies involving cell culture, nucleic acid and protein isolation and for setting up in vitro amplification procedures. The third laboratory is for conducting experiments where PCR product analysis, molecular cloning and protein analysis, immuoblot and ELISA analysis are performed. Each laboratory has its own dedicated equipments. Moreover, chemicals, necessary reagents and consumables are maintained separately in each laboratory.

Offices: We have three 150 sq. ft. offices for use by the research personnel. The department has a well-organized office constituting several secretarial staff and accountants who are readily available to support the needs of our projects.

Computers: Several personal computers are installed in all of the offices and in laboratories. All of the computers have ether net connections for the direct high speed access to the Internet. In addition, they have the complete access to the Unix computer system provided by the KSU. The Unix main frame computer is loaded with the latest release of Genetic Computer Group (GCG) programs for molecular biology studies. In addition, our college has a multimedia center that is a useful source for technical support and troubleshooting.

Major Equipment in my laboratory: The equipment in Dr. Ganta's laboratories include an electroporator, two conventional 96 tube capacity PCR machines (a Perkin-Elmer GenAmp 9700 and an Eppindorf temperature gradient PCR with an *in situ* PCR module), and a Smart Cycler system and a BioRad iCycler system for real time PCR/RT-PCR analysis, manual and automated DNA sequencing apparatus, several agarose and poly acrylamide gel systems, a pulsed field gel apparatus, a 2D gel electrophoresis system for protein, DNA and RNA analysis, a hybridization oven, a blot transfer apparatus, a UV cross linker, Kodak image analysis system, several water baths, shaker incubators, a speed-vac system, a sequencing gel drier, several micro centrifuges, a sonicator, four CO_2 incubators, two sterile cell-culture hoods, two cyto-centrifuges, a sero-centrifuge, three fume hoods, a cryogenic freezer, three chromatography refrigerators, three freezers, several liquid nitrogen tanks, several weighing balances (micro and macro), three refrigerated high speed centrifuges, and pH meters. We are also equipped with a fluorescence microscope having the capability to record images using digital imaging system.

We also have the complete access to many shared facilities. They include several additional PCR machines, X-ray film developers, ice machines, scintillation counters, autoclaves, and a centralized deionized water supply. We also have a dark room that contains a UV light/Polaroid camera assembly, a walk in cold room, a 37^0C incubator, and an instrumentation room containing several commonly used equipment such as the high speed/ultra centrifuges. These are located within the close proximity of my laboratories. Our department also has an automated DNA sequencer, a transmission and scanning electron microscope, and Becton Dickinson FACScan for flow cytometry analysis. Our college has a core facility for confocal miscroscopic studies.

The Kansas State University has several biotechnology core facilities to support research on genomics, functional genomics and molecular biology. The facilities include several state-of-the-art equipment, such as the MALDI-TOF and LC-MS/MS mass spectrometry instruments and protein/peptide sequencing equipment (http://www-personal.ksu.edu/~jtomich/corelab.html).

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Roman	Reddy	Ganta	

Position/Title: Professor

Department: Diagnostic Medicine/Pathobiolo

Organization Name: Kansas State University

Division: College of Veterinary Medicine

* Street1: 1800 Denison Avenue

Street2:

* City: Manhattan

County: Riley

* State: KS: Kansas

Province:

* Country: USA: UNITED STATES

* Zip / Postal Code: 66506

*Phone Number	Fax Number	* E-Mail
785-532-4612	785-532-4039	rganta@vet.ksu.edu

Credential, e.g., agency login: eRA Commons User Name

*** Project Role:** PD/PI

Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	5485-Ganta-NIH-format_CV07.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

Redacted by agreement

Obtained via FOIA by White Coat Waste Project

Redacted by agreement

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

Additional Senior/Key Person Form Attachments

When submitting senior/key persons in excess of 8 individuals, please attach additional senior/key person forms here. Each additional form attached here, will provide you with the ability to identify another 8 individuals, up to a maximum of 4 attachments (32 people).

The means to obtain a supplementary form is provided here on this form, by the button below. In order to extract, fill, and attach each additional form, simply follow these steps:

- Select the "Select to Extract the R&R Additional Senior/Key Person Form" button, which appears below.
- Save the file using a descriptive name, that will help you remember the content of the supplemental form that you are creating. When assigning a name to the file, please remember to give it the extension ".xfd" (for example, "My_Senior_Key.xfd"). If you do not name your file with the ".xfd" extension you will be unable to open it later, using your PureEdge viewer software.
- Using the "Open Form" tool on your PureEdge viewer, open the new form that you have just saved.
- Enter your additional Senior/Key Person information in this supplemental form. It is essentially the same as the Senior/Key person form that you see in the main body of your application.
- When you have completed entering information in the supplemental form, save it and close it.
- Return to this "Additional Senior/Key Person Form Attachments" page.
- Attach the saved supplemental form, that you just filled in, to one of the blocks provided on this "attachments" form.

Important: Please attach additional Senior/Key Person forms, using the blocks below. Please remember that the files you attach must be Senior/Key Person Pure Edge forms, which were previously extracted using the process outlined above. Attaching any other type of file may result in the inability to submit your application to Grants.gov.

1) Please attach Attachment 1	
2) Please attach Attachment 2	
3) Please attach Attachment 3	
4) Please attach Attachment 4	

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)

Filename

MimeType

Additional Biographical Sketch(es) (Senior/Key Person)

Filename

MimeType

Additional Current and Pending Support(s)

Filename

MimeType

Obtained via FOIA by White Coat Waste Project

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Roman R. Ganta	**Professor**
eRA COMMONS USER NAME eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Andhra University, Waltair, India	B. S.	1975-78	Zoology, Botony, Chemistry
Andhra University, Waltair, India	M.S.	1978-80	Biochemistry
All India Institute of Medical Sciences, New Delhi, India	Ph.D.	1982-87	Biochemistry
The Scripps Research Institute, La Jolla, CA	Postdoctoral	1987-90	Molecular Biology
University of Florida, Gainesville, FL	Postdoctoral	1990-92	Molecular Parasitology

Please refer to the application instructions in order to complete sections A, B, and C of the Biographical Sketch.

A. Positions and Honors.

Positions and Employment

1992-98 Assistant Scientist, Dept. Pathobiology, University of Florida, Gainesville, FL.
1998-03 Assistant Professor, Department of Diagnostic Medicine/Pathobiology, Kansas State University.
2003-2007 Associate Professor, Department of Diagnostic Medicine/Pathobiology, Kansas State University.
2007- Professor, Department of Diagnostic Medicine/Pathobiology, Kansas State University.

Other Experience and Professional Memberships

American Society for Microbiology: Member, 2001 to current
American Society for Rickettsiology: Member, 1998-current; Secretary/Treasurer, 2003- current.

Honors

Pfizer Animal Health Award for Research Excellence: Recipient of the Pfizer Animal Health Award for Excellence in Research by a Faculty Member in 2005, College of Veterinary Medicine, Kansas State University, Manhattan, KS.

NIH Study section: Ad-hoc member on Bacteriology and Mycology 1 study section for the years 2003 and 2004 and IDMA study section in 2005.
Member, Vector Biology Study Section, NIH peer review panel, 2007-2011.

Oklahoma Center for the Advancement of Science and Technology (OCAST), Oklahoma City: Grants Review panel member for the OCAST since 2002. (Appointed in 2005 to serve as the Chairperson for the Infectious Diseases/Immunology grants review panel.)

B. Selected peer-reviewed publications (Note: Name in some papers appears as G. R. Reddy**):**

1. **G. R. Reddy**, and A. Ramaiah. Purification of rabbit liver phosphofructokinase and its properties under simulating *In Vivo* conditions. *J. Biosci*. 6, 643-653, (1984).
2. J. W. Harvey and **G. R. Reddy**. Postnatal hematological development in phosphofructokinase-deficient dogs. *Blood*, 74, 2556-2561, (1989).
3. P. M. Sharma, **G. R. Reddy**, S. Vora, B. M.Babior and A. McLachlan. Cloning and expression of the human muscle phosphofructokinase cDNA. ***Gene* 77**, 177-183, (1989).
4. P. M. Sharma, **G. R. Reddy**, B. M. Babior, and A. McLachlan. Alternative splicing of the transcript encoding the human muscle isozyme of phosphofructokinase. *J. Biol. Chem.,* 265, 9006-9010, (1990).
5. **G. R. Reddy**, D. Chakrabarti, C. A. Yowell and J. B. Dame. Sequence microheterogeneity of the three small subunit ribosomal RNA genes of *Babesia bigemina*: Expression in erythrocyte culture. *Nucl. Acids Res*. 19, 3641-3645, (1991).

Obtained via FOIA by White Coat Waste Project

6. **G. R. Reddy** and J. B. Dame. Ribosomal RNA based method for sensitive detection of *Babesia bigemina* in bovine blood.. ***J. Clin. Microbiol.*** **30**, 1811-1814, (1992).
7. **G. R. Reddy**, P. A. Vasanen, P. M. Sharma, R. Kohani, J. L. Johnson, A. K. Raney, B. M. Babior and A. McLachlan. Cloning and characterization of the human muscle phosphofructokinase gene. *DNA and Cell Biology* 11, 461-470, (1992).
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44. Unpublished
45. Unpublished

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C. Research Support: Ongoing Research Support:

NIH-R01: AI55052 9/30/03 to 1/31/08
Title: Cellular immunity against Ehrlichia chaffeensis. The goals of this project are to evaluate the contributions of macrophages, T-cells and cytokines to the clearance of E. chaffeensis.
PI: R.R. Ganta

NIH-NCRR P20 RR17686 09/30/02 – 06/30/07
Title: Epithelial Function in Health and Disease. The goal of this project is to establish a Center of Excellence for the investigation of the function of epithelial cells. The primary purpose of the Center will be to develop the research careers of basic scientists and clinician scientists at the three institutions.
PI: D. Marcus (PI). Role on the project for Ganta: Director for the Molecular Biology Support Core.

NIH-R13: AI071693 07/15/2006 - 06/30/2007
Title: American Society for Rickettsiology (ASR) conference 2006. The goals of this project are to support the ASR conference to be held in Asilamor, California, September 2-7, 2006.
PI: R.R. Ganta

Completed during the last three years:

Private Source 9/01/01 to 8/31/03
Title: Molecular detection of single or co-infections with five known canine ehrlichiosis agents. Goal of this project is to develop a multiplex molecular diagnostic test for the simultaneous detection of up to five pathogens of the genera Ehrlichia and Anaplasma.
PI: R.R. Ganta

NIH-NCRR P20 RR17686 09/30/02 – 06/30/05
Title: Epithelial Function in Health and Disease. The goal of this project is to establish a Center of Excellence for the investigation of the function of epithelial cells. The primary purpose of the Center will be to develop the research careers of basic scientists and clinician scientists at the three institutions.
PI: D. Marcus. Role on the project for Ganta: Junior Investigator (2002-03) and Mentor (2004-05).

NIH-R13: AI66872 06/01/05 – 05/30/06
Title: American Society for Rickettsiology (ASR) conference. The goals of this project are to support the ASR conference held in Spain, 2005.
PI: R.R. Ganta

Kansas State Department of Health and Environment 6/01/2002 to 06/30/05
Title: Laboratory analysis West Nile Virus surveillance in Kansas. The goal of this project is to conduct molecular evaluation of the spread of the West Nile Virus in the state of Kansas.
PI: R.R. Ganta

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of the Freedom of Information and Privacy Act

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PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Roman
Middle Name: Reddy
* Last Name: Ganta
Suffix:

* New Investigator? ● No ○ Yes

Degrees: MS | PhD |

2. Human Subjects

Clinical Trial? ● No ○ Yes

* Agency-Defined Phase III Clinical Trial? ○ No ○ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Mr.
* First Name: Paul
Middle Name: R.
* Last Name: Lowe
Suffix:

* Phone Number: 785-532-6804
Fax Number: 785-532-5944
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* Title: Asst. Vice Provost for Research

* Street1: PreAward Services
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* City: Manhattan
County: Riley
* State: KS: Kansas
Province:
* Country: USA:
* Zip / Postal Code: 66506-1103

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PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 9/30/2007

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ●No ○Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/registry/index.asp . Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

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PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001
Expiration Date: 9/30/2007

Budget Period: 1

Start Date: 12/01/2007 End Date: 11/30/2008

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	MTDC	46.00	250,000.00	115,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) Department of Health & Human ServicesJames R. Nolan(214) 767-3529

Indirect Cost Rate Agreement Date 05/16/2003

Total Indirect Costs 115,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 365,000.00

Budget Period: 2

Start Date: 12/01/2008 End Date: 11/30/2009

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	MTDC	46.00	115,000.00	115,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) Department of Health & Human ServicesJames R. Nolan(214) 767-3529

Indirect Cost Rate Agreement Date

Total Indirect Costs 115,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 365,000.00

Obtained via FOIA by White Coat Waste Project

PHS 398 Modular Budget, Periods 3 and 4

OMB Number: 0925-0001
Expiration Date: 9/30/2007

Budget Period: 3

Start Date: 12/01/2009 End Date: 11/30/2010

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	MTDC	46.00	250,000.00	115,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) Department of Health & Human ServicesJames R. Nolan(214) 767-3529

Indirect Cost Rate Agreement Date 05/16/2003

Total Indirect Costs 115,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 365,000.00

Budget Period: 4

Start Date: 12/01/2010 End Date: 11/30/2011

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	MTDC	46.00	250,000.00	115,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) Department of Health & Human ServicesJames R. Nolan(214) 767-3529

Indirect Cost Rate Agreement Date 05/16/2003

Total Indirect Costs 115,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 365,000.00

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Tracking Number:

PHS 398 Modular Budget, Period 5 and Cumulative

OMB Number: 0925-0001
Expiration Date: 9/30/2007

Budget Period: 5

Start Date: 12/01/2011 End Date: 11/30/2012

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	MTDC	46.00	250,000.00	115,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) Department of Health & Human ServicesJames R. Nolan(214) 767-3529

Indirect Cost Rate Agreement Date 05/16/2003

Total Indirect Costs 115,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 365,000.00

Cumulative Budget Information

1. Total Costs, Entire Project Period

* Section A, Total Direct Cost less Consortium F&A for Entire Project Period	$	1,250,000.00
Section A, Total Consortium F&A for Entire Project Period	$	
* Section A, Total Direct Costs for Entire Project Period	$	1,250,000.00
* Section B, Total Indirect Costs for Entire Project Period	$	575,000.00
* Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period	$	1,825,000.00

2. Budget Justifications

Personnel Justification	8428-Personnel_Justification2.pdf
Consortium Justification	
Additional Narrative Justification	

Obtained via FOIA by White Coat Waste Project

Attachments

PersonnelJustification_attDataGroup0

File Name	Mime Type
8428-Personnel_Justification2.pdf	application/pdf

ConsortiumJustification_attDataGroup0

File Name	Mime Type

AdditionalNarrativeJustification_attDataGroup0

File Name	Mime Type

Obtained via FOIA by White Coat Waste Project

Personnel Justification

Roman R. Ganta, M.S., Ph.D., Principal Investigator EFFORT He will be responsible for overseeing all aspects of the project. A microbiologist, a post-doctoral associate, and two graduate students will work under his direct supervision.

Redacted by agreement Ph.D., Professor and Collaborator Redacted by agreement will serve as the collaborator on this project. He has been an active collaborator on the development of preminary data and will continue the collaboration in the proposed project. His input is sought during the planning and execution of promoter mapping analysis, including the designing of mutation constructs. He also serves as a Redacted by agreement

Redacted by Ph.D., Collaborator Redacted by agreement will serve as the collaborator on this project. His input is sought during the planning and execution of the transformation and mutation experiments, including the designing of transformation constructs. He also provides the plasmid constructs prepared in his laboratory.

Post-doctoral Associate (12.0/ Cal.) A post-doc will be employed to perform experiments proposed in the project.

Redacted by agreement, Microbiologist III EFFORT Redacted by will assist with culturing E. chaffeensis, transformation experiments proposed in Aim 3 and 4 and the routine laboratory maintenance. She will work in collaboration with the graduate students and post-doctoral associate.

Two Ph.D. Students: Two graduate students with 6.0/ Cal. appointment will work on the project on the regulation of gene expression and genetic manipulation experiments. The students already generated several parts of the preliminary data in support of this project.

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OMB Number: 0925-0001
Expiration Date: 9/30/2007

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

❍New ● Resubmission ❍ Renewal ❍ Continuation ❍ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	2553-Ganta-Introduction_review-response_.pdf
2. Specific Aims	1320-Ganta-Specific-aims.pdf
3. Background and Significance	2042-Ganta-Background&Significance.pdf
4. Preliminary Studies / Progress Report	6562-Ganta-Preliminary_data.pdf
5. Research Design and Methods	7989-Ganta-Research_design_and_methods.pdf
6. Inclusion Enrollment Report	
7. Progress Report Publication List	

Human Subjects Sections

Attachments 8-11 apply only when you have answered "yes" to the question "are human subjects involved" on the R&R Other Project Information Form. In this case, attachments 8-11 may be required, and you are encouraged to consult the Application guide instructions and/or the specific Funding Opportunity Announcement to determine which sections must be submitted with this application.

8. Protection of Human Subjects	
9. Inclusion of Women and Minorities	
10. Targeted/Planned Enrollment Table	
11. Inclusion of Children	

Other Research Plan Sections

12. Vertebrate Animals	
13. Select Agent Research	
14. Multiple PI Leadership	
15. Consortium/Contractual Arrangements	
16. Letters of Support	5654-Ganta-support_letters.pdf
17. Resource Sharing Plan(s)	

18. Appendix

Obtained via FOIA by White Coat Waste Project

Attachments

IntroductionToApplication_attDataGroup0

File Name	Mime Type
2553-Ganta-Introduction_review-response_.pdf	application/pdf

SpecificAims_attDataGroup0

File Name	Mime Type
1320-Ganta-Specific-aims.pdf	application/pdf

BackgroundSignificance_attDataGroup0

File Name	Mime Type
2042-Ganta-Background&Significance.pdf	application/pdf

ProgressReport_attDataGroup0

File Name	Mime Type
6562-Ganta-Preliminary_data.pdf	application/pdf

ResearchDesignMethods_attDataGroup0

File Name	Mime Type
7989-Ganta-Research_design_and_methods.pdf	application/pdf

InclusionEnrollmentReport_attDataGroup0

File Name	Mime Type

ProgressReportPublicationList_attDataGroup0

File Name	Mime Type

ProtectionOfHumanSubjects_attDataGroup0

File Name	Mime Type

InclusionOfWomenAndMinorities_attDataGroup0

File Name	Mime Type

TargetedPlannedEnrollmentTable_attDataGroup0

File Name	Mime Type

InclusionOfChildren_attDataGroup0

File Name	Mime Type

VertebrateAnimals_attDataGroup0

File Name	Mime Type

SelectAgentResearch_attDataGroup0

File Name	Mime Type

MultiplePILeadershipPlan_attDataGroup0

File Name	Mime Type

ConsortiumContractualArrangements_attDataGroup0

File Name	Mime Type

LettersOfSupport_attDataGroup0

File Name	Mime Type
5654-Ganta-support_letters.pdf	application/pdf

ResourceSharingPlans_attDataGroup0

File Name	Mime Type

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Appendix

File Name	Mime Type
3796-Ganta-Appendix_3.pdf	application/pdf
6257-Ganta-Appendix_4.pdf	application/pdf
5385-Appendix_Material.pdf	application/pdf

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INTRODUCTION:

reviewers' comments

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reviewers' comments

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SPECIFIC AIMS: Vector-borne pathogenic bacteria reside in the complex cellular environment of vectors and in vertebrates with sophisticated systems of defense. However, the pathogenic bacteria have developed strategies to evade host defenses in support of their continued growth. For example, *Ehrlichia chaffeensis,* an emerging tick-borne pathogen responsible for human monocytic ehrlichiosis (HME), causes persistent infection in its hosts (10,23,25,26,29,46). Disease manifestation of HME varies significantly from an asymptomatic infection to a severe, life threatening disease with a mortality rate of about 3% of the reported cases. The relationship between *E. chaffeensis* and its target host cells (macrophages/monocytes and tick cells) is critical for the survival of the organism, as it must persist in the target host cells to complete its life cycle. *E. chaffeensis* differs significantly in macrophages and tick cells, having distinct patterns of protein expression (89,90) (Appendix 1 and 2). A multigene locus containing 22 genes that encode for the 28 kDa outer membrane proteins (p28-Omp) has been reported for *E. chaffeensis* and for other closely related species (18,44,65,74-76,99,105). The differentially expressed proteins of *E. chaffeensis* in macrophage and tick cells include those from the p28-Omp locus (89,98). The gene expression from this locus is primarily restricted to the p28-Omp gene 19 in macrophages. In tick cells, the expressed protein is from the p28-Omp 14 gene. Our studies on several isolates of *E. chaffeensis* and another macrophage-tropic *Ehrlichia*, *E. canis*, also suggest the host-specific protein expression is conserved, including those from the p28-Omp locus (90). In addition, we discovered that the differences in protein expression are the result of differences in transcription levels. We also reported recently that host clearance is delayed for *E. chaffeensis* originated from tick cells as compared to macrophage derived bacteria in the murine host (34) (Appendix 3). However, a significant gap of knowledge exists in understanding the mechanisms by which *E. chaffeensis* regulates its gene expression to alter protein expression in arthropod and vertebrate host environments. Also, it is entirely unclear how the organisms respond to the loss of gene activity from differentially expressed genes.

To date, only a few studies have described the transfer of genetic material into *rickettsiales*. In the absence of a method useful to introduce mutations in the genome, characterizing a gene function or the regulation of gene expression is challenging. *In vitro* transcription can serve as a possible alternative means to understanding the regulation of gene expression as reported for *Chlamydia trachomatis* (57,79,101). In this study, we will examine the *in vitro* methods to map the regulation of gene expression as well as to develop a method to assess the impact of gene knockouts on the pathogen's survival in vertebrate and tick cell environments. In our preliminary studies, we developed methods of *in vitro* transcription coupled translation system to map *E. chaffeensis* promoters. This newly developed molecular tool forms the basis for conceiving the current project to evaluate differential gene expression in *E. chaffeensis*. We also have made considerable progress in establishing the protocols to introduce gene disruption mutations. The present study aims to address significant gaps in knowledge about gene regulation mechanisms and their biological significance in *Ehrlichia* by studying a set of differentially expressed genes in response to two different host cell environments. The central hypothesis is that the transcription from the p28-Omp multigene locus in *E. chaffeensis* is differentially regulated in response to environmental signals and that the host cell-specific gene expression is essential for the pathogen's survival in vertebrate and tick cell environments. The goals of the project will be met by performing experiments to address the following four specific aims.

Specific Aim 1: Establish *in vitro* transcription system for mapping *Ehrlichia* promoters.
Specific Aim 2: Map transcriptional machinery in differentially expressed genes 14 and 19 of the p28-Omp locus.
Specific Aim 3: Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19.
Specific Aim 4: Evaluate knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific expression.

The results of this study will provide novel information in understanding *Ehrlichia* gene regulation, how the tick-borne pathogen in macrophages and tick cells respond to the loss of differentially expressed genes. The regulation of gene expression is a fundamental process that controls protein synthesis and alterations in protein expression in *Ehrlichia* in response to vertebrate and invertebrate host systems. To the best of our knowledge, this will be the first study that is focused to understand the regulation of gene expression in tick-transmitted pathogens belonging to the genera *Ehrlichia* and *Anaplasma*. We will also be the first to assess the loss of a gene function in tick-transmitted *rickettsiae* belonging to the *Anaplasmataceae* family. The outcomes of this study will have much broader implications for controlling tick-borne illnesses caused by the emerging rickettsial pathogens in people.

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BACKGROUND AND SIGNIFICANCE:

The family *Anaplasmataceae* contains several obligate, intracellular, Gram-negative bacteria that include species of the genera *Ehrlichia* and *Anaplasma* (28,82). A steady increase of potentially fatal human diseases caused by the species of the genera *Ehrlichia* and *Anaplasma* that infect phagocytic cells has been reported in recent years (27,61,84). These include two recently identified tick-transmitted emerging diseases of humans: human monocytic ehrlichiosis (HME) caused by *Ehrlichia chaffeensis* (22,54) and human granulocytic anaplasmosis (HGA) caused by *Anaplasma phagocytophilum* (16,20). *Ehrlichia ewingii*, another tick-transmitted agent, has also been reported to be infectious to people (13). Tick-transmitted pathogens, including *E. chaffeensis*, must develop strategies to persist in both tick and vertebrate hosts in order to successfully complete their life cycle. These strategies are particularly important for a pathogen, such as *E. chaffeensis,* which cannot be transovarially transmitted from an adult female tick to its offspring (28,80,82). Unlike insect vectors, ticks typically have an extremely low rate of success in finding a host for a blood meal. Specifically, the probability of transmitting a pathogen from a tick to a vertebrate host would be significantly low. Likewise, it is critical for a tick-transmitted pathogen to survive in the vertebrate host for extended periods of time. Therefore, it is necessary for tick-transmitted pathogens, such as *E. chaffeensis*, to have evolved strategies to establish persistent infections in both vertebrate and tick hosts successfully to complete their lifecycle. In fact, it is widely documented that tick-transmitted rickettsiales of the genera *Ehrlichia* and *Anaplasma*, including *E. chaffeensis,* establish persistent infections (2,21,23,24,29,32,93,98,107,110).

It is critical to understand how tick-transmitted *Anaplasmataceae* organisms remain in their tick vectors and vertebrate hosts for extended periods of time. There may be many ways by which the organisms may persist. The strategies may include altering the host response, varying the expressed antigens relative to time post-infection, and differential host-specific antigenic expression. As ticks are heterothermic and vertebrate hosts are homeothermic, *E. chaffeensis* may also have evolved to express unique antigens in response to their host environments. We recently demonstrated that the rapid clearance of *E. chaffeensis* requires the activation of macrophage and helper T-cells (35,36). Studies reported in the literature also suggest that the B-cell activation, including the p28-outer membrane antigen-specific responses contributes significantly to the clearance of the pathogen from a vertebrate host (48,49,103). We presented evidence that *Ehrlichia* species alter the expression of many proteins when replicating in vertebrate and invertebrate host cells (89, 90). In a follow up study, we reported that clearance of the pathogen by the murine host is delayed for *E. chaffeensis* grown in tick cells as compared with those grown in vertebrate host cells (34). Furthermore, the host response differed considerably from *E. chaffeensis* originating from tick and vertebrate host environments. Much of the fundamental knowledge about the host-specificity and how it contributes to the pathogen's persistence can be understood by understanding the regulation of gene expression in *Ehrlichia*.

A limited number of studies have been carried out on *E. chaffeensis* in an effort to examine the contributions of expressed antigens in support of persistent infection. Studies on the antigenic profiles of *E. chaffeensis* identified many immuno reactive antigens, including several outer membrane antigens (17,81). Among these is a group of proteins having molecular size of approximately 28 kDa. These antigens have epitopes that are cross-reactive with antibodies of several related species (18,44,105). The p28 outer membrane protein (p28-Omp) gene locus in *E. chaffeensis* contains 22 tandemly arranged paralogous genes that differ mostly in three variable regions where immunogenic epitopes are located (19,65,74,75,106). These structural features, and the presence of variable domains having B-cell epitopes in the p28-Omp locus, have generated considerable interest for their possible role in the pathogen's persistence.

Several recent studies that evaluated the transcripts of the p28-Omp locus from *E. chaffeensis* in both *in vitro* and *in vivo* by non-quantitative RT-PCR methods have offered conflicting results and suggesting that it is difficult to draw meaningful conclusions when assessed by *in vitro* amplification methods of RNA analysis (16,19,41,64). We resolved this controversy by determining the presence of transcripts with more direct methods, such as the Northern blot, primer extension, RNase protection and microarray analysis (34,89,90) (described in preliminary studies section). Furthermore, we conducted proteomics studies to identify expressed antigens from the p28-Omp locus in *Ehrlichia* grown in macrophages and tick cells (89,90). Our studies demonstrated that the major expression from the p28-Omp locus is limited only to a subset of genes and the antigens expressed from this locus are unique to vertebrate and tick cells. Our results also suggest that the expression sites in macrophages and tick cells are also conserved in several *E. chaffeensis* isolates and *E. canis,* a closely related macrophage-tropic pathogen (90).

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The p28-Omp antigens may be essential components for the pathogen's survival in adapting to multiple hosts and to establish persistent infection. The adoptive transfer of antibodies against the p28-Omp antigens of *E. chaffeensis* is protective against fatal infection in SCID mice (49). However, the mice become moribund after withdrawal of the adoptive transfer of antibodies suggesting the importance of other immune components, such as T-cells. We recently reported the importance of helper T-cells in conferring protection against *E. chaffeensis* (35). The evaluation of a p28-Omp gene-based DNA vaccine for *Ehrlichia ruminantium* suggests that the gene product provides protection against a fatal infection (63). The protein coding sequences of the p28-Omp genes of *Ehrlichia* species share significant homology to the outer membrane protein gene families of *Anaplasma* species, MSP2 and p44 (36). Recent data suggest that the MSP2 and p44 gene loci of *Anaplasma* species contribute in generating antigenic variation during infection (7,8,9,12,59). These antigens are also involved in inducing T-cell response (47). Expression of unique variants from these gene families is reported for *Anaplasma* species in their tick and vertebrate hosts, although the gene expression sites remained unaltered (9,12,39). The protein coding sequences of the p28-Omp genes share structural similarity to the variant outer surface protein antigen gene loci of *Borrelia* species and *Neisseria gonorrhoea* (41,88,108). Taken together, these similarities suggest that the proteins made from the p28-Omp multigene locus of *E. chaffeensis* are important components, possibly for the pathogen's survival in vertebrate and tick hosts. The demonstration of host cell-specific differential protein expression and the existence of various glycosylated and phosphorylated forms (89,90) suggest that *Ehrlichia* species may have evolved to include multiple levels of regulation which may occur at transcription, translation, and post-translation levels and may aid in the pathogens' survival in vertebrate and tick hosts.

An infectious disease is the result of a pathogen's ability to overcome host clearance. Understanding the complexity of an infection in a host cell is essential in devising strategies to control a disease. In our study, we will conduct experiments to gain fundamental knowledge important for understanding the regulation of gene expression by *E. chaffeensis* in vertebrate and invertebrate host environments. Transcription analysis *in vitro* using *Ehrlichia* RNA polymerase or with an *E. coli* system can serve as a good strategy to define the molecular basis for altered gene expression in response to host environments. We expect that *in vitro* analysis will help in understanding gene regulation and the contributions of the host environment in influencing the *Ehrlichia* gene expression. For example, studies have shown that *in vitro* transcription to be a valuable tool in mapping gene function and to map the promoters of various genes in another intracellular bacterium, *Chlamydia trachomatis* (57,79,87,95,96,101). In our preliminary studies, we developed several protocols to support the goals to map transcription and translation for differentially expressed *E. chaffeensis* genes. These tools will allow us to evaluate *E. chaffeensis* gene expression that is influenced by different host cell backgrounds.

Genetic manipulation systems can be used to define the functional significance of niches used by *E. chaffeensis* for its survival in vertebrate and tick hosts. Currently, research on *E. chaffeensis* is hampered by the lack of protocols for conducting genetic transformation and mutational analysis. This proposed study therefore, attempts to fill in the important gap of knowledge in *E. chaffeensis* research, specifically by developing molecular tools needed to introduce mutations in this pathogen. Importantly, we will generate mutations in the *E. chaffeensis* genome to assess the impact of the loss of differentially expressed genes for the pathogen's survival in tick and vertebrate host environments. Tools developed in this study will also be valuable in characterizing the regulation of gene expression for other *Anaplasmataceae* pathogens.

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PRELIMINARY STUDIES:

We generated considerable data to provide several lines of evidence for the differential host cell-specific protein expression. In addition, we established numerous protocols necessary for accomplishing the goals of this study. These protocols include the demonstration of *in vitro* transcription and translation assays, promoter activity for genes 14 and 19, Northern blot, primer extension, RNase protection, gel mobility shift, real time RT-PCR, and Western blot assays. In support of specific aim 4 (new to this submission), several plasmid constructs were made for use in generating partial or complete gene duplication or gene disruption mutations by homologous recombination at a genomic region spanning a gene from the p28-Omp locus that is not one among differentially expressed genes. Parts of the preliminary data have been published recently (34,89,90) (Appendix 1-3) and additional data will soon be published in manuscripts currently in preparation. Here, we presented a brief summary of the published data and detailed description of unpublished data in support of Specific Aims 1-4.

Proteome analysis identifies unique macrophage and tick cell-specific protein expression by *E. chaffeensis*: Extensive proteome analysis was performed to map and identify expressed antigens of *E. chaffeensis* grown in vertebrate and invertebrate host environments (89,90). Our studies revealed many macrophage- and tick cell-specific differentially expressed proteins in *E. chaffeensis*. Proteome maps of differentially expressed proteins were very similar for *E. chaffeensis* that was replicating in several macrophage-derived cell lines. Likewise, the pathogen proteomes in two different tick cell lines were highly homologous. Differentially expressed proteins included those expressed from the p28-Omp multigene locus (89,90). The p28-Omp expressed proteins of the pathogen in various macrophage cell lines remained the same and were primarily the product of the gene 19, whereas in tick cells (originating from both vector and non vector ticks) it was the product of gene 14. The p28-Omp expressed protein was the product of the p28-Omp14 gene product. The p28-Omp genomic locus and the host cell-specific proteins expressed from this locus were identified in a cartoon form in **Figure 1**.

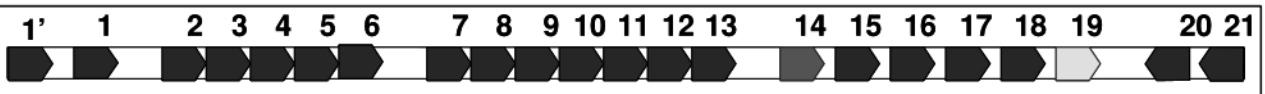

Figure 1: P28-Omp locus and macrophage-specific and tick cell-specific expressed genes were identified in yellow and red color boxes, respectively.

Evaluation of the transcriptome of *E. chaffeensis* in vertebrate and tick host cells: The proteome studies described above suggest that *E. chaffeensis* makes different p28-Omp proteins in macrophages and tick cells. However, RNA analysis by RT-PCR revealed conflicting results (16,41,64). Specifically, previous studies suggest the expression of multiple transcripts and inconsistencies in the detected transcripts. These observed discrepancies in transcription and translation may be explained if 1) *E. chaffeensis* has two levels of control; one at transcription and the other at translation. This may indicate that not all transcripts from the p28-Omp locus are translated into proteins, or 2) the level of transcription and translation varies for different genes of the p28-Omp locus. ***In our more recent studies, we pursued more sensitive mass spectrometry methods such as the Matrix Assisted Laser Desorption Ionization Time-of-flight (MALDI-TOF) and the large-scale proteome analysis by Multidimensional Protein Identification Technology (MudPIT) (100). The MudPIT method has the ability to detect and identify proteins rarely seen using other proteome analysis tools (100). These additional methods aided in the detection of many new and low abundant proteins. The data remained consistent with the previous mass spectrometry analysis that the p28-Omp expressed protein from tick cell-derived E. chaffeensis was from gene 14 while in the bacteria originating from macrophage has the predominant expression from p28 -Omp 19 gene with minor expression from several other p28-Omp genes (manuscript in preparation).***

We evaluated the presence of transcripts for a subset of genes by more direct detection methods; Northern blot, primer extension and RNase protection analysis. The whole genome microarray was developed and array analysis was performed to map the expressed transcripts from the p28-Omp locus. Finally, transcription levels were assayed by diplex real time RT-PCR assay. Parts of these data were published recently and the remaining data are currently being compiled as a manuscript for publication (will be submitted soon). These data are summarized below.

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Northern blot analysis: Northern blot analysis was performed using standard molecular protocols (83) using 10 µg of *E. chaffeensis* RNA recovered from bacteria grown in macrophage (DH82) and tick cells (ISE6) resolving on a denaturing gel, transferred to a nylon membrane and probed with ^{32}p labeled DNA probes specific for genes p28-Omp 14 or 19 (**Figure 2**). The analysis revealed the presence of the p28-Omp 19 transcript in macrophage-grown *E. chaffeensis;* whereas RNA from tick cell-grown *E. chaffeensis* contained transcripts only for the gene 14 (90).

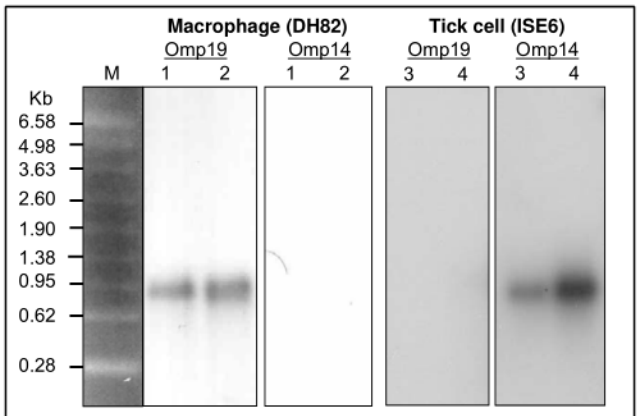

Figure 2: Northern blot analysis. About 10 µg each of two independent batches of *E. chaffeensis* RNA isolated from cultures grown in macrophage cell line, DH82 (lanes 1 and 2) and tick cell line, ISE6 (lanes 3 and 4) were resolved in duplicate sets on a denaturing RNA gel and assessed by Northern blot analysis using p28-Omp 14 or 19 gene-specific 32P-labeled probes. The experiment was repeated twice using independently isolated batches of RNA. The gene 19 transcripts of the size 0.9 kb were detectable only in macrophage-derived RNA samples. Similarly, the tick cell-derived RNA samples contained 0.9 kb transcripts for the gene 14. RNA molecular weight markers were resolved in lane M.

Primer extension: Primer extension analysis helps to define the transcription start site as well as to assess the presence of transcripts for a given gene (14). About 10 µg of RNA isolated from macrophage or tick cell-grown *E. chaffeensis* was assessed by primer extension. Gene 14 and 19 specific complimentary primers were prepared and end labeled with γ-^{32}p ATP using T4 polynucleotide kinase. The labeled primers were annealed to RNA and the primer extension was performed using a primer extension kit (Promega Corp, Madison, WI). The products from this reaction were resolved in a denatured 6% polyacrylamide sequencing gel. The presence of radiolabelled primer extended c-DNA products in the resolved gel was detected by exposing it to an X-ray film. The data presented in **Figure 3** revealed the presence of a 177 bases long fragment only for the p28-Omp gene 14 in RNA obtained from *E. chaffeensis* grown in tick cells. In macrophage grown *E. chaffeensis*, RNA contained primer extended products of about 270 bases long for the p28-Omp gene 19. This experiment also allowed us to identify the transcription start sites for the genes 14 and 19. They are 35 bases upstream for gene 14 and ~46 bases upstream for the gene 19 to the first base of the coding sequence. A faint signal for gene 19 was also detectable in tick-cell derived *E. chaffeensis* RNA that corresponds to a 270 bases long fragment suggesting that this gene is also transcriptionally active in tick cell-derived *E. chaffeensis*, but level of expression is very low.

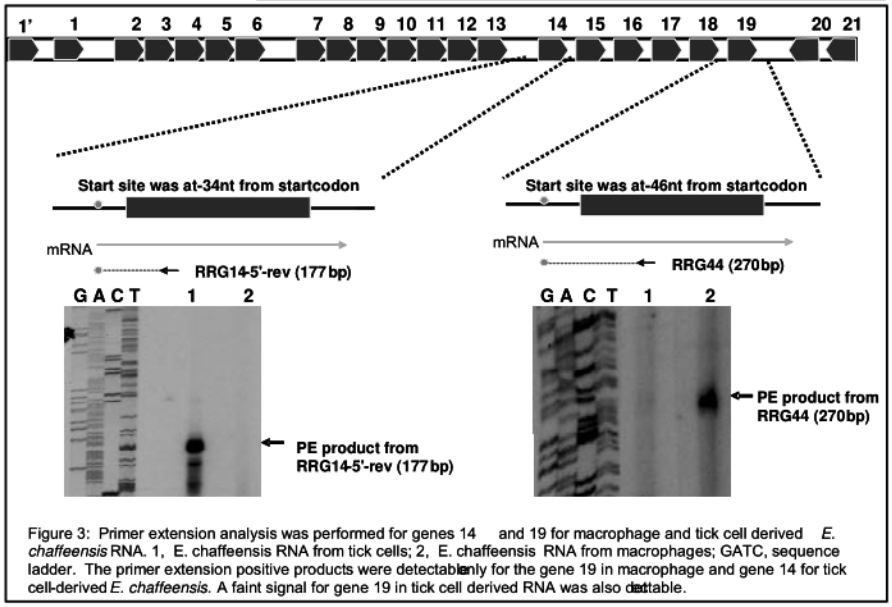

Figure 3: Primer extension analysis was performed for genes 14 and 19 for macrophage and tick cell derived *E. chaffeensis* RNA. 1, E. chaffeensis RNA from tick cells; 2, E. chaffeensis RNA from macrophages; GATC, sequence ladder. The primer extension positive products were detectable only for the gene 19 in macrophage and gene 14 for tick cell-derived *E. chaffeensis*. A faint signal for gene 19 in tick cell derived RNA was also detectable.

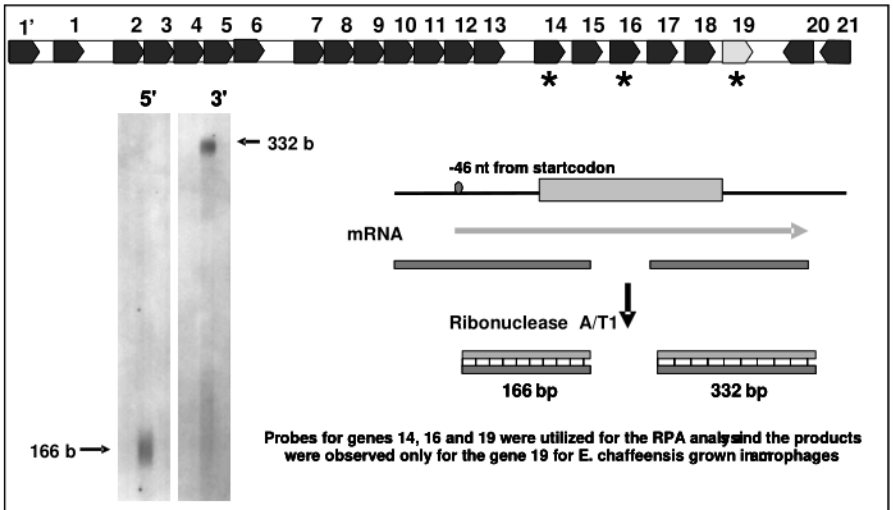

Figure 4: Ribonuclease Protection Assay. RNase protection assay was performed on macrophage-derived E. chaffeensis RNA using 5' and 3' end-specific probes for genes 14 and 19, and with a 3' specific probe for gene 16. The p28-Omp locus, the schematic representation of the experiment and protected fragments detected for gene 19 on a sequencing gel were shown. The experiment revealed no protected fragments for genes 14 and 16.

RNase Protection assay: RNase protection assay (RPA) is another useful method to assess the true presence or absence of transcripts for a given gene (83). RPA was performed only for macrophage derived *E. chaffeensis* RNA. Ten µg of RNA was assessed by RNase

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protection assay using RNase protection kit (Roche Applied Sciences, Indianapolis, IN). Gene-specific complementary DIG-labeled RNA probes were generated by PCR and by *in vitro* transcription strategies (two fragments each spanning the 5' and 3' ends of the p28-Omp 14 and 19 genes and one 3' segment for p28-Omp16). The probes were used to hybridize with the *E. chaffeensis* RNA and the hybridization mixture was subjected to RNase T1 digestion to remove all unbound, single stranded RNA with the exception of protected parts of the mRNA. The protected fragments were then detected after being resolved on a denatured 6% polyacrylamide gel, transferred to a nylon membrane and developed with antidigoxigenin alkaline phosphatase conjugate and chemiluminescent substrate, CSPD. The data presented in **Figure 4** revealed the presence of protected fragments of transcripts for gene 19 in *E. chaffeensis* grown in macrophages. No protected fragments were detectable for genes 14 or 16. Protected fragments at the 5' end allowed us to verify the transcription start site for genes19. The transcription start site was the same site detected in the primer extension analysis of gene 19. The 3' end RNase protection assay further verified the results regarding the presence of gene 19 transcripts.

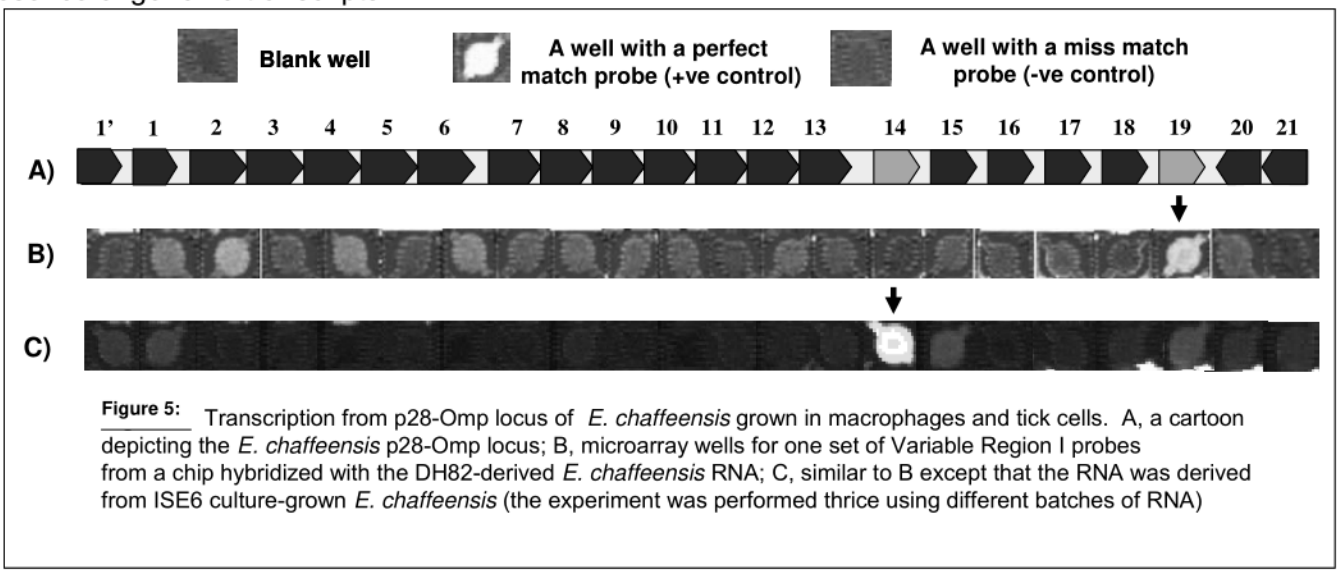

Figure 5: Transcription from p28-Omp locus of *E. chaffeensis* grown in macrophages and tick cells. A, a cartoon depicting the *E. chaffeensis* p28-Omp locus; B, microarray wells for one set of Variable Region I probes from a chip hybridized with the DH82-derived *E. chaffeensis* RNA; C, similar to B except that the RNA was derived from ISE6 culture-grown *E. chaffeensis* (the experiment was performed thrice using different batches of RNA)

Whole genome microarrray: Although the primer extension data are in agreement with the Northern blot and RNase protection experiments, the appearance of very faint band for gene 19 in the tick cell derived *E. chaffeensis* RNA raises the possibility that transcripts are also made for other genes, but at low levels. The direct RNA analysis experiments are limited to only three genes analyzed in our studies. To examine if other genes from the p28-Omp locus are transcriptionally active, we developed a whole genome microarray. We obtained the unannotated, complete genome sequence of *E. chaffeensis* from The Institute for Genomic Research (TIGR) and carefully analyzed for the presence of open reading frames (ORFs) with the aid of the Artemis genome sequence analysis program (Welcome Trust Sanger Institute, Cambridge, UK). A total of 1,234 ORFs were identified and the probes for microarray were designed for all ORFs. The annotated *E. chaffeensis* whole genome sequence is published recently (42). In the annotated sequence, 1,115 ORFs were reported. We predicted about 119 more ORFs than those reported in the annotated sequence. For each gene, we prepared two 45mer-long oligonucleotides. In addition, two to four probes each for all 22 genes of the p28-Omp locus were selected from the three variable regions. We custom ordered the arrays from Xeotron corporation (now affiliated with Invitrogen, Carlsbad, CA). The probes were spotted in triplicates and distributed randomly throughout the microarray chip (38).

Host cell-free *Ehrlichia* organisms released from cultures grown to about 90-100% infectivity were recovered by performing differential centrifugation and ultra filtration methods. Total RNA was isolated using Tri reagent method (Sigma-Aldrich, St. Louis, MO). Purified RNA was passed through RNeasy mini elute columns (Qiagen, Valencia, CA) to remove residual genomic DNA. The integrity, purity and concentration of RNA samples were assessed by using Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The chip hybridization and initial output data analysis were performed at the Vanderbilt University Microarray Shared Resource Facility (Nashville, TN). About 4 µg of RNA was reverse transcribed using superscript II (Invitrogen, Carlsbad, CA) in the presence of random hexamers, chip control spikes, and amino allyl modified

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dUTPs (Sigma-Aldrich, St. Louis, MO). The amino allyl-labeled targets were then coupled to aliquots of ester linked Cy3 or Cy5 dyes (GE Healthcare, Piscataway, NJ). The labeled targets were used to hybridize the microarray chips for 18 h at 35°C on M-2 microfluidic station and washed as specified by the manufacturer (Invitrogen, Carlsbad, CA). Arrays were scanned to visualize the labeled wells using a GenePix 4000B scanner (Molecular Devices Corporation, Union City, CA). To evaluate the reproducibility of the data from microarray analysis, we performed self to self hybridization of the macrophage (DH82), or ISE6 tick cell culture-derived *E. chaffeensis* RNA labeled with Cy3 and Cy5. The data were analyzed using Gene Traffic (Stratagene, La Jolla, CA) and MS Excel (Microsoft, Redmond, WA). The intensity of fluorescence observed from the background, empty wells and controls i.e., spots with mismatched probes was subtracted from the total fluorescence intensity observed from each spot. The spots with fluorescence intensities below a threshold value were not considered for analysis. This threshold value was determined for each hybridization-based on the intensity observed in the control spots in that chip. The average fluorescence observed from all the spots for a particular gene was used to determine if that gene was expressed or not (Manuscript describing the whole genome microarray is in preparation.) Microarray data for one probe each originating from Variable Region I of p28-Omp genes for all 22 genes for both macrophage- and tick cell-derived RNA are presented in **Figure 5**. Major expression was detected in the microarray wells of gene 14 in ISE6 ticks cell-derived RNA, while macrophage-derived RNA had predominant transcripts for gene 19. Signals in the microarray wells, particularly for the macrophage-derived *E. chaffeensis* RNA, were also visible for several other genes, but at lower intensities than those for the p28-Omp gene 19 transcripts. Signal intensities detected in wells representing all 22 genes were plotted to determine the quantitative differences in the levels of gene expression (**Figure 6**). The data reflect findings from three independent experiments. Signals for Variable Region I probes were plotted against gene numbers, and the average values of expression were presented with error bars. These microarray analyses further supported the differential host cell-specific expression from genes 14 and 19 of the p28-Omp locus. The RNA analysis data reported in the literature (16,41,64) and the presence of minor signals for several genes of the p28-Omp locus observed in microarray suggest that several genes of the p-28 Omp locus are transcriptionally active, but levels of transcription vary in different genes. If the variations in transcription are responsible for differential expression from genes 14 and 19, we will be able to demonstrate the presence of varying levels of transcripts by a more sensitive real-time RT-PCR assay (53,94).

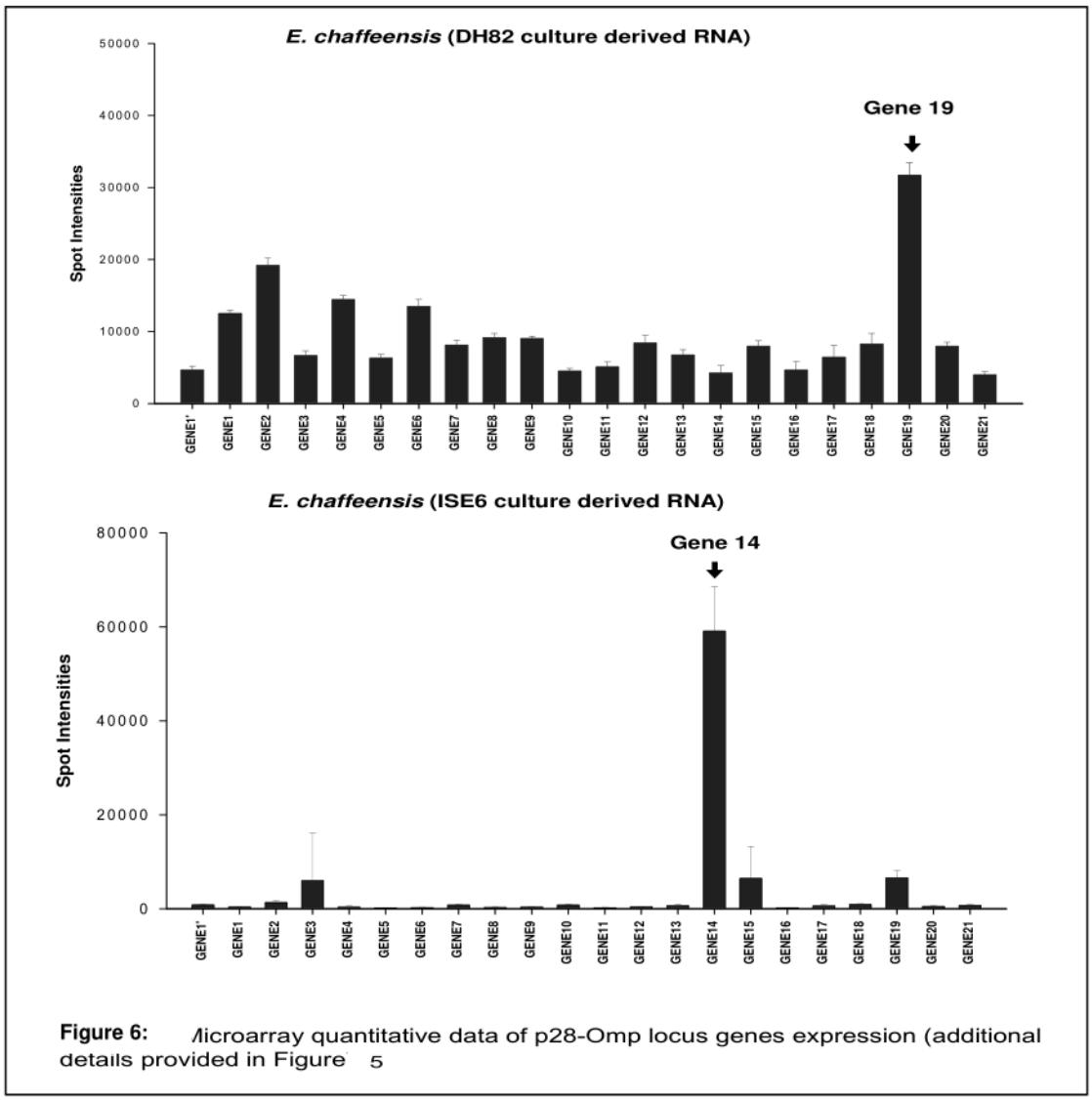

Figure 6: Microarray quantitative data of p28-Omp locus genes expression (additional details provided in Figure 5

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Real-time RT-PCR to monitor transcription from genes 14 and 19: We designed a diplex TaqMan real time RT-PCR assay to detect the variations in the mRNA levels for genes 14 and 19 using a similar strategy reported in (91). To examine variations in the levels of transcription, we compared expression from *E. chaffeensis* p28-Omp genes 14 and 19 in RNA isolated from tick cell and macrophage cultures using diplex RT-PCR assay (**Figure 7**) (90). The assay was performed in the presence or absence of a reverse transcriptase. The experiment was repeated two additional times and statistical analysis was performed (**Table 2**). Because RNA preparation typically contains genomic DNA, diplex PCR results will allow us assess the true presence of transcripts for genes 14 and 19. As only one gene copy each is present in the genome; we expect to obtain identical Ct-values for DNA (Ct-values refer to the PCR cycle at which the PCR machine detects fluorescence levels above a threshold level which is template dependant). However, Ct-values for RNA should be lower than the DNA Ct-value. Additionally, if there is a variation in the levels of transcription for genes 14 and 19 in response to the growth in different host cell backgrounds, it should be evident from the Ct-values observed in the presence of reverse transcriptase.

Table 2: Differential expression of the genes p28-Omp 14 and 19 of *E. chaffeensis* cultured in tick cells (ISE6) and macrophages (DH82).

Host cell background	Assay	Gene 14	Gene 19	p Value
Macrophages (DH82)	PCR	23.85±0.74	23.8±0.89	0.47024
	RT-PCR	20.86±0.25	16.23±0.21	0.00001*
Tick cells (ISE6)	PCR	25.98±1.27	25.73±1.37	0.41429
	RT-PCR	18.86±0.4	24.73±0.49	0.00005*

* p values of <0.001 (Student t- test)

The diplex real time RT-PCR assay performed in the presence or absence of reverse transcriptase more accurately represents the expression of p28-Omp genes than the use of a house keeping gene. The following conclusions were drawn from this experiment: 1) The Ct-values for DNA for both genes are essentially identical for all samples analyzed, thus confirming that the values represent data for DNA; 2) The Ct values in the presence of reverse transcriptase for genes 14 and 19 are always lower compared to Ct-values in the absence of reverse transcriptase, suggesting that both the genes are transcriptionally active in *E. chaffeensis* grown in macrophages and tick cells; 3) The average difference in the Ct values of genes for genes 19 to 14 (gene 19 being lower) from three independent experiments was 4.99 cycles for *E. chaffeensis* RNA isolated from macrophages. This corresponds to 31.77 fold more gene 19 compared with gene 14 in the *E. chaffeensis* grown in macrophages and 4) The average difference in the RNA Ct values of gene 14 to 19 RNA isolated from *E. chaffeensis* grown in tick cells was 4.56 cycles (gene 14 being lower). This difference corresponds to 23.58 fold more RNA expression from gene 14 compared to gene 19 in tick cell-grown *E. chaffeensis*. These results demonstrate that both genes 14 and 19 of the p28 Omp locus are transcriptionally active independent of the host environment in which the pathogen is grown. However, the macrophage and tick cells have a significant impact in altering the pathogen's transcription levels. Specifically, the transcription from gene 14 is either suppressed or alternatively gene 19 transcription is enhanced under macrophage environment and the opposite is true when the pathogen is replicating inside the tick cells. Tick cell-specific expression from p28-Omp 14 was also confirmed.

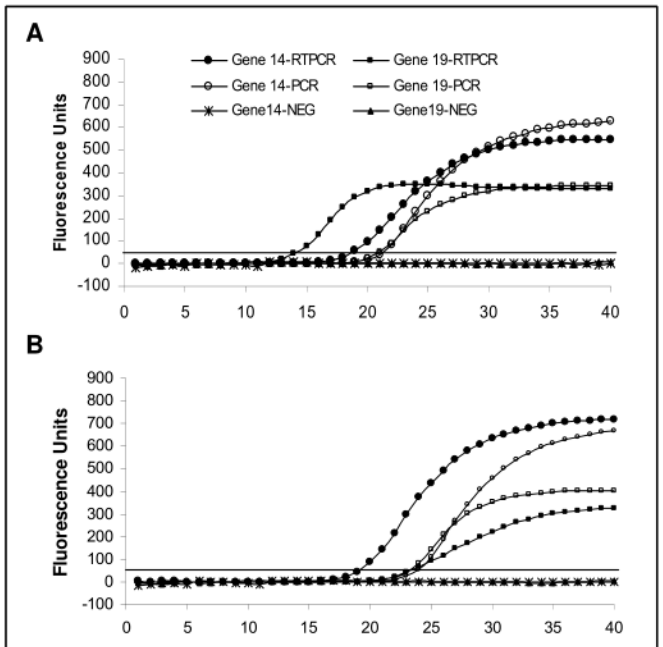

Figure 7: Diplex real-time RT-PCR assay. TaqMan-based diplex real time RT-PCR analysis was performed using total RNA isolated from infected macrophages (DH82) (panel A) or tick cells (ISE6) (panel B). The amplification cycles were plotted against fluorescence emission for the analysis performed in the presence or absence of reverse transcriptase. A horizontal solid line in panel on the X axis depicts the line for threshold fluorescence. Fluorescence emission crossing this line at an amplification cycle is regarded as the Ct value. (Gene 14-NEG and Gene 19-NEG represent data derived for reaction negative control diplex assay that included all assay components but no template; Gene 14-RTPCR and Gene 19-RTPCR represent data derived for a reaction containing RNA in presence of reverse transcriptase; Gene 14-PCR and Gene 19-PCR represent data generated for RNA from the diplex assay that did not include reverse transcriptase. The analysis was repeated twice using independently isolated batches of RNA.

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in a cell line derived from the *E. chaffeensis* vector tick (*A. americanum*). [All these data were described in our recent publication (90) (Appendix 2)].

The exciting data described above is the basis for our hypothesis that **transcription from p28-Omp multigene locus is differentially regulated in response to environmental signals coming from macrophages and tick cells**. To test this hypothesis, a method to map the transcription in *E. chaffeensis* is needed. Specifically, a method should be established to investigate a question: how is the regulation of gene expression in *E. chaffeensis* accomplished in response to different environmental cues? This enquiry may be addressed by mapping the transcription using an *in vitro* assay similar to that employed for *C. trachomatis* (58,78,102). We performed several additional experiments in support of the development of an *in vitro* transcription system. These include constructing plasmids that contained putative promoter regions of the genes 14 and 19, assessing their promoter activity in *E. coli* under *in vitro* conditions and the developing of an *in vitro* assay that could evaluate transcription from *E. chaffeensis* genes. These experiments were described below.

Promoter constructs of p28 Omp 14 and 19: Promoter constructs using lacZ as a reporter gene: Sequences downstream from the termination codon of gene 13 to upstream of the initiation codon of gene 14 were amplified from *E. chaffeensis* genomic DNA. Similarly, to obtain gene 19 putative promoter sequences a primer set was designed and used to amplify sequence downstream from the termination codon of gene 18 and upstream of the initiation codon of gene 19. The PCR products were ligated and cloned into the promoterless pBlue-TOPO cloning vector, containing lacZ reporter gene (Invitrogen Technologies, Carlsbad, CA). One clone each in forward and reverse orientations were selected for the gene14 (pBlue-TOPO14F or R) and 19 (pBlue-TOPO19ForR). In addition, a promoterless self ligated plasmid was selected to serve as a negative control (the cloning strategy is outlined in **Figure 8**).

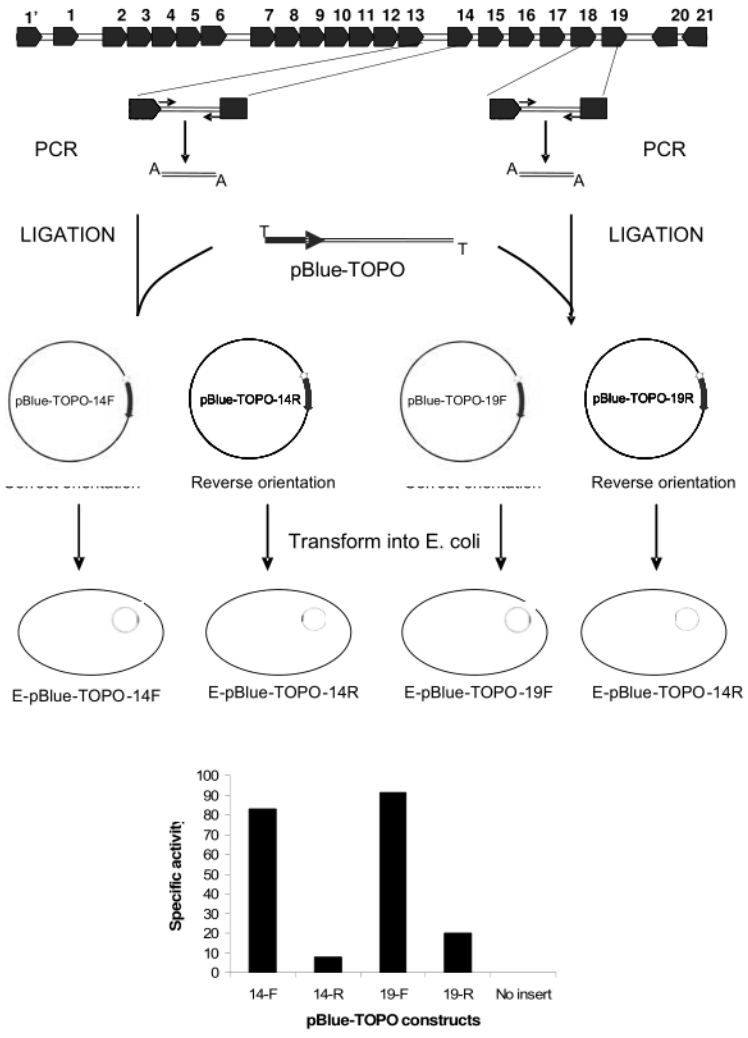

Figure 8. Construction of lacZ plasmids containing gene 14 and 19 putative promoters. **A)** The promoter regions upstream for the genes were amplified and ligated into the pBlue-TOPO vector that contains the promoterless reporter gene LacZ. Plasmid containing inserts were selected after transforming into *E. coli* strain, TOP10. **B)** Constructs in both orientations were selected for the analysis. In addition, no insert control was selected to serve as the negative control. β-galctosidase enzyme activity is assessed in the *E. coli* extracts prepared from all five constructs and the specific activiy of the enzyme was assessed after performing the enzyme assay and measuring the color development at 414 nm as per the manufacturer's instructions and the data were presented after correcting the background values for the construct contained no insert (negative control). Lysates prepared from *E. coli* containing the gene 14 and 19 promoters in correct orientation had higher levels of enzyme activity. Promoters ligated into the opposite orientation are not expected to serve the promoters to drive β-galctosidase expression. The background activity in these reverse orientation constructs (10-20%) may represent the non-specific binding of the *E. coli* RNA polymerase to the reverse orientation DNA of the promoters located upstream to the LacZ gene.

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The recombinant colonies of *E. coli* containing all five plasmids were grown to an optical density of 0.4. The cell lysates were prepared to determine the β-galactosidase activity using ONPG (Ortho-Nitrophenyl-β-D-Galactopyranoside) as a substrate as per the kit protocol (Invitrogen Technologies, Carlsbad, CA). The β-galactosidase activity was detected in all *E. coli* extracts. However, *E. coli* containing constructs having putative promoters of genes 14 and 19 in the correct orientation had higher levels of β-galactosidase activity. The constructs containing the putative promoters in reverse orientation or promoter less lacZ gene construct contained low level background activity. These data provide the first evidence that the sequences upstream from the genes 14 and 19 are active in *E. coli* suggesting that *E. coli* RNA polymerase recognizes *Ehrlichia* promoters.

Promoter constructs using GFP as a reporter: To verify the above results, we obtained a GFP promoterless construct (pPROBE-NT) reported by Miller et al. (62). Validity of this plasmid to support the promoter activity for an *Anaplasmataceae* pathogen, *Anaplasma marginale*, has been reported recently by Barbet *et al* (6). We obtained the pPROBE-NT vector (a gift from Dr. Steven Lindow, University of California, Berkeley, CA) and the putative promoter sequences for genes 14 and 19 were cloned. Recombinants containing the promoters of genes 14 and 19 are expected to express GFP only if the *E. coli* RNA polymerase drives the expression from these promoters. GFP positive colonies were observed in recombinants containing gene 14 or 19 promoters, but not in the promoterless pPROBE -NT (**Figure 9**). To further verify these observations, *E. coli* lysates were prepared, resolved on a 12 % polyacrylamide gel and Western blot analysis was performed using GFP polyclonal antibody (Rockland Immunochemicals, Inc. Gilbertsville, PA) (**Figure 10**). We detected GFP protein in the clones containing putative promoter for gene 14 and 19. Taken together, these data confirmed our observations using the lacZ reporter based constructs that *E. coli* RNA polymerase can serve as a surrogate system to drive the transcription from *Ehrlichia* promoters. The activity of the gene 14 and 19 promoters in *E. coli* was also reported earlier by Long et al. (52), thus validating the proposed experiments.

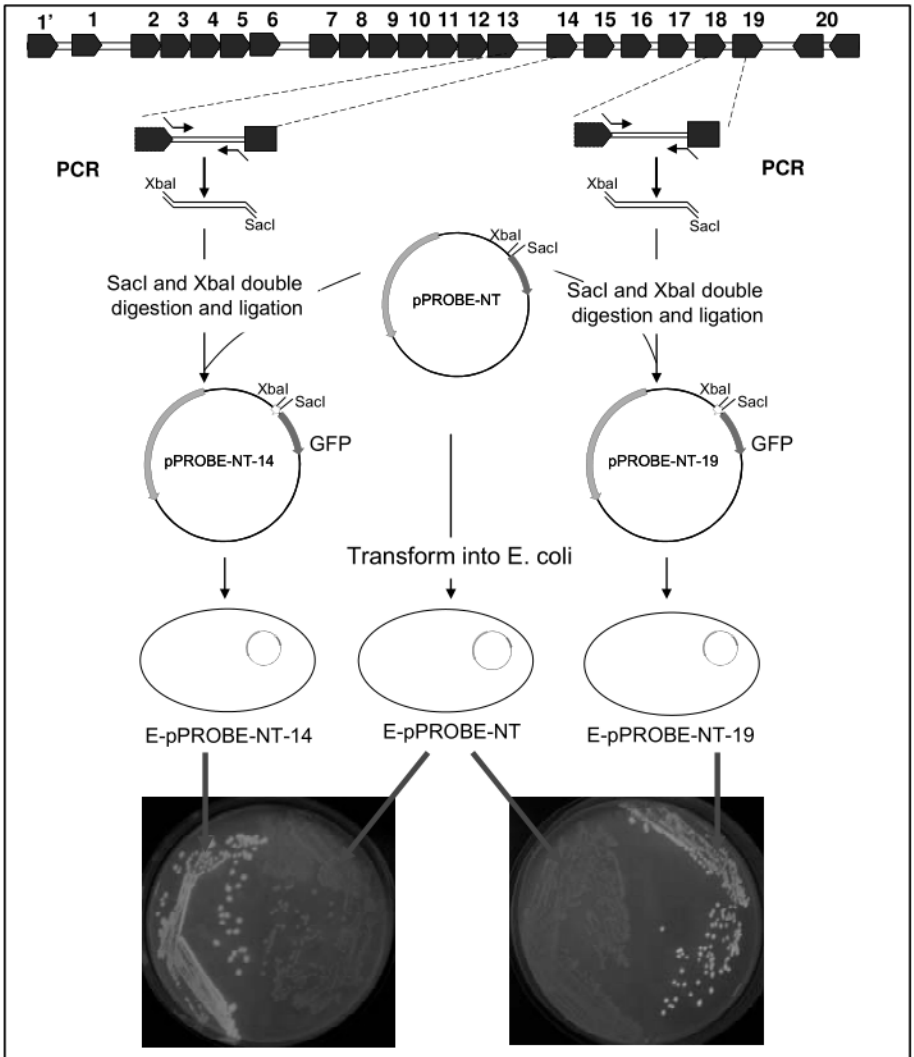

Figure 9: Construction of GFP expressing plasmids containing the genes 14 and 19 promoters: Two sets of primers were designed for amplifying the putative promoter regions of genes 14 and 19. Restriction sites for XbaI and SacI were engineered into the primers to aid in the directional cloning. The primers were used to amplify the promoter regions using *E. chaffeensis* genomic DNA as the template. The amplicons were double digested with SacI and XbaI and ligated into the SacI and XbaI double digested pPROBE-NT plasmid. The plasmids containing inserts were selected after transformation into *E. coli* strain, XL1Blue (Stratagene, La Jolla, CA). GFP positives were detected for recombinants in *E. coli* for the promoter constructs.

***In vitro* transcription assay using *E. coli* extracts:** An *in vitro* transcription assay for the use in mapping *Ehrlichia* promoters was also developed. Twenty ng each of plasmids p-PROBE-NT14 or pPROBE-NT19 or pPROBE-NT or

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without a plasmid and 35 µl of *E. coli* extracts (Novagen, Madison, WI) (to serve as the source of RNA polymerase) and all ingredients necessary for transcription and translation were added to a final volume of 50 µl reaction. The reaction mixture was incubated at 37°C for 90 min. At the end of the reaction, 10 µl each were resolved on a polyacrylamide gel, and assessed for the presence of GFP using polyclonal sera against GFP protein. The GFP protein was detected in reactions containing GFP fusion plasmids having genes 14 or 19 promoters. Promoterless GFP plasmid and *E. coli* extract alone did not produce GFP protein (**Figure 11**). These data provide evidence that the appropriate conditions for *in vitro* transcription and translation systems are now available for further studies using *E. coli* RNA polymerase.

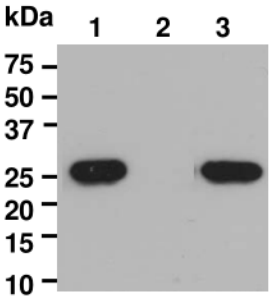

Figure 10. Western blot analysis to determine the GFP expression in *E. coli* containing genes14 and 19 promoter constructs. *E. coli* lysates were prepared to examine the GFP expression in *E. coli* containing gene 14 promoter containing plasmid (pPROBE-NT14) (lane 1), promoterless GFP plasmid (pPROBE-NT) (lane 2), and gene 19 promoter containing plasmid (pPROB-NT19) (lane 3). Approximately 25 µg of protein was resolved on a 12 % polyacrylamide gel, transferred to a nitrocellulose membrane and assessed for the presence of GFP using polyclonal sera against the GFP protein (Rockland, Gilbertsville, PA). A 25 kDa GFP protein is detectable only in the *E. coli* lysates containing genes 14 and 19 promoter plasmids. Molecular weight markers were shown to identify the size.

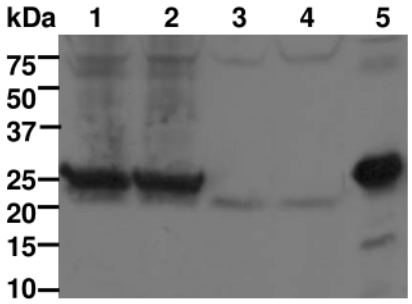

Figure 11. In vitro transcription and translation assay using E. coli RNA polymerase. The plasmids pPROBE-NT14 (lane 1), pPROBE-NT19 (lane 2), or pPROBE-NT (lane 3), and *E. coli* extracts in a final volume of 50 µl reaction were incubated at 37°C for 90 min. Similarly, *E. coli* extracts alone were incubated (lane 4). At the end of the reaction, 10 µl each were resolved on a 12% polyacrylamide gel, transferred onto nitrocellulose membrane and assessed for the presence of GFP using polyclonal sera against GFP protein (Rockland, Gilbertsville, PA). The GFP protein was detectable in lanes 1 and 2 that contained the genes14 and 19 promoters in front of the GFP reporter gene. Promoterless GFP reporter gene plasmid and *E. coli* extract alone did not produce a GFP protein. Lane 5 contains lysates from *E. coli* containing pPROBE-NT14 plasmid to serve as the positive control.

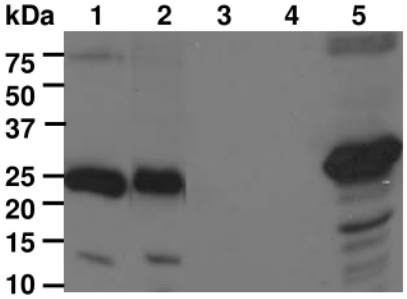

Figure 12. In vitro transcription and translation assay to examine if lysates prepared from *E. chaffeensis* can drive GFP expression. *E. chaffeensis* host cell-free organisms were prepared from 25 ml culture and suspended in 2 ml lysis buffer, sonicated for 4 times, centrifuged to remove the cell debris. Supernatant was used as the source of RNA polymerase for in vitro transcription and translation assay. Twenty ng of plasmids each of the pPROBE-NT14, pPROBE-NT19, or pPROBE-NT, and 35 µl of *E. chaffeensis* lysate were added to 50 µL reactions containing buffers. The samples were incubated at 37°C for 90 min. Ten µL each of the reaction products were resolved on a 12% polyacrylamide gel, transferred to a nitrocellulose membrane, and Western blot analysis was performed to detect the GFP expression. Lane 4 contained only *E. chaffeensis* lysates and buffers. GFP expression was detectable only in lanes containing the reaction products with the genes 14 and 19 promoter constructs (lanes 1, 2), but not in promoterless GFP reporter gene plasmid (lane 3) or *E. chaffeensis* extracts alone (lane 4). Lane 5 contains extracts from *E. coli* containing pPROBE-NT19 to serve as the positive control for GFP in the Western blot.

***In vitro* transcription/translation assay using *E. chaffeensis* extracts:** In this experiment, we tested the hypothesis that *E. chaffeensis* lysate, contains all the necessary ingredients to perform *in vitro* transcription coupled translation assay. In our earlier studies, we prepared *E. chaffeensis* lysates that contained DNA binding proteins, useful in evaluating DNA-protein interactions by the gel mobility shift assay (74) (Appendix 4). We used the same protocol to prepare *E. chaffeensis* extracts to use in the current *in vitro* transcription/translation assay. Three separate reactions containing 20 ng each of the plasmids pPROBE-NT14 or pPROBE-NT19 or pPROBE-NT, and 35 µl of *E. chaffeensis* lysate in a total volume of 50 µl containing a reaction buffer [150 mM Tris-HCl (pH 8.0), 100 mM KCl, 10 mM magnesium acetate, 1 mM EDTA, 5 mM DTT, and 10% glycerol] was incubated for 90 min at 37°C. Another reaction contained *E. chaffeensis* lysate and buffers but no template. At the end of reaction 10 µl each of the reaction products were resolved on a polyacrylamide gel, transferred to a nitrocellulose membrane, and Western blot analysis was performed to detect different GFP expression using polyclonal sera described as above (**Figure 12**). GFP expression was detected only in plasmids containing genes 14 and 19 promoters (lanes 1, 2). These data are very exciting and demonstrate that the lysate prepared from *E. chaffeensis*, may prove valuable in studies evaluating the promoter activity of *E. chaffeensis* genes under *in vitro*. Taken together, these studies demonstrate that we have the necessary protocols and reagents to further develop in vitro transcription system and map differential regulation of p28-Omp genes of *E. chaffeensis*.

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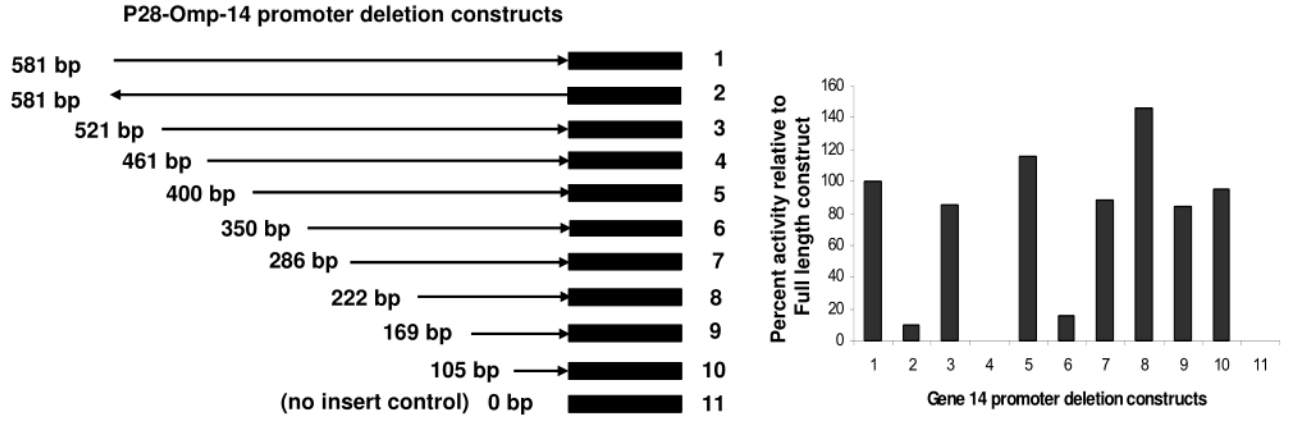

Figure 13: Deletion analysis of the gene 14 promoter construct. Deletion constructs for gene 14 promoter were prepared by PCR and cloning strategies using primers internal to the gene 14 upstream sequences. β-galctosidase gene expression in the extracts prepared from the *E. coli* cultures of bacteria transformed with different constructs were determined by ELISA assays with 5 µg of protein each, specific activity for each construct was estimated. Percent of activity for each construct was expressed relative to the enzyme activity detected for the full length construct (activity for full length is taken as 100%). Deletion constructs and their orientations were illustrated as a cartoon on the left and the relative enzyme activities were presented as bar graphs on the right.

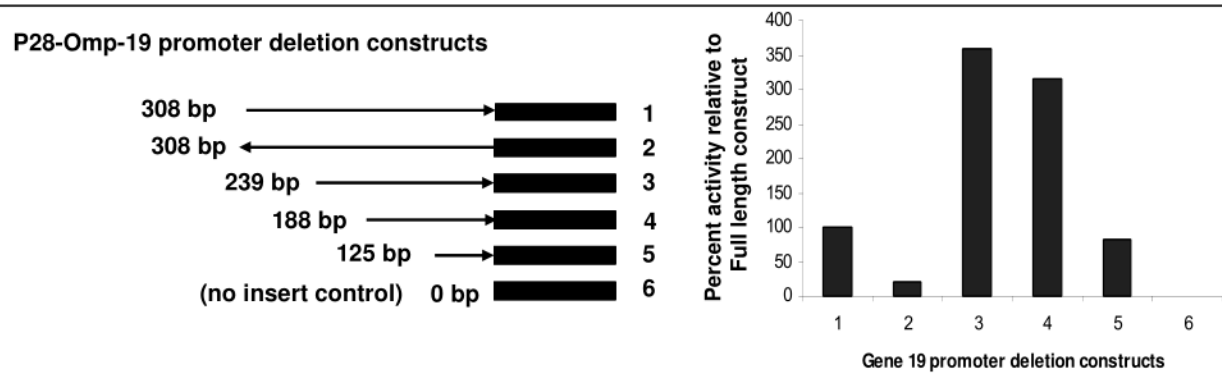

Figure 14: Deletion analysis of the gene 19 construct. Preparation of deletion constructs and the analysis were performed as described above in Figure 13. The gene 19 construct had fewer deletion constructs as the full length insert size is about 280 bases smaller.

Preliminary evidence suggesting that the p28-Omp 14 and 19 promoter regions contain transcription regulators: In an effort to identify minimal promoters and to locate regions involved in the regulation of gene expression, we performed several deletion constructs for genes 14 and 19 and assessed their promoter activities. Several serial deletion constructs with 5' end deletions ranging from 60 to 476 bp for gene 14 promoter and 69 to 183 bp for gene 19 promoter were prepared (Figure 13 and 14). The constructs prepared in pBlue-TOPO were used for evaluating the promoter activity by assessing the β-galctosidase expression. The constructs in the reverse orientation with the complete upstream sequences for genes 14 and 19 were also included. The β-galctosidase expression was determined and expressed as percent activity relative to the full length promoter activity for all of these constructs. All of the deletion constructs in the correct orientation exhibited promoter activity (**Figure 13 and 14**). In addition, the level of activity was different for different deletion constructs (in some, the activity was higher while for others it was lower compared to the full length constructs). Together, these data suggest that the deleted segments contain regulatory elements that are probably involved in accomplishing host-specific differential expression.

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Preliminary data in support of introducing mutations in *E. chaffeensis* (Specific Aim 4; new to this submission):

Preparation of constructs for p28-Omp gene 15 mutations: Our proteome and transcriptome analysis suggests that the p28-Omp 15 gene is transcriptionally silent/or expressed at very low levels in *E. chaffeensis* grown in macrophage or tick cell. We hypothesize that the disruption of this gene in the genome will not have detrimental effects on the growth of *E. chaffeensis.* As it is also possible that this gene may be critical for the pathogen survival, we also planned to create mutations that allow gene 15 duplication or partial gene 15 insertion. Therefore, in support of the first set of experiments, we prepared plasmid constructs for generating insertion mutations (1) by homologous recombination to generate gene 15 duplication or a partial gene 15 insertion mutations in *E. chaffeensis*. A strategy was also developed similarly to prepare a gene disruption mutation in the *E. chaffeensis* gene 15. We selected the rifampin resistance gene as the antibiotic selection marker, as it has been reported that *E. chaffeensis* is sensitive to rifampin at concentrations of 0.1 µg/ml or higher (11). *E. chaffeensis* sensitivity was also verified in our laboratory.

Molecular strategies were employed for preparing plasmid constructs as outlined below and shown in **Figure 15**. Plasmid, Blue Script SK$^+$ (pBS), and *E. coli,* XL1 Blue strain (Stratagene, La Jolla, CA) were used to prepare the constructs. First, we prepared a pBS lacking EcoR I and Hind III sites from the multiple cloning sites region of the pBS. (It was necessary to remove these restriction sites in support of subsequent steps of manipulation to prepare homologous recombination constructs.) Two PCRs were performed using *E. chaffeensis* genomic DNA plus primers that will allow the amplification of either the p28-Omp 15 complete coding sequence plus 5' and 3' flanking sequences or partial gene 15 coding sequence (lacking 136 bases at the 5' end of the ORF) plus 3' flanking sequence. The amplicons containing the complete gene 15 (referred to as 15C) or a partial gene 15 (named as 15P) were cloned into the EcoR V site (for blunt end ligation) of the pBS, EI$^-$, HIII$^-$ plasmid. The clones were referred to as pBS-15C or pBS-15P (**Figure 15**). The rifampin resistance gene that has the *rpsL* promoter of *R. prowazekii* from the pMW1409 plasmid was amplified using the proof reading enzyme, *pfu* ultra DNA polymerase (Stratagene, La Jolla, CA), primers and the pMW1409 plasmid were described in (68). (The pMW1409 was kindly provided by our collaborator, Dr. Redacted Redacted The PCR was ligated into the Sma I site (blunt end ligation) of the pBS-15C or pBS-15P plasmid to generate the

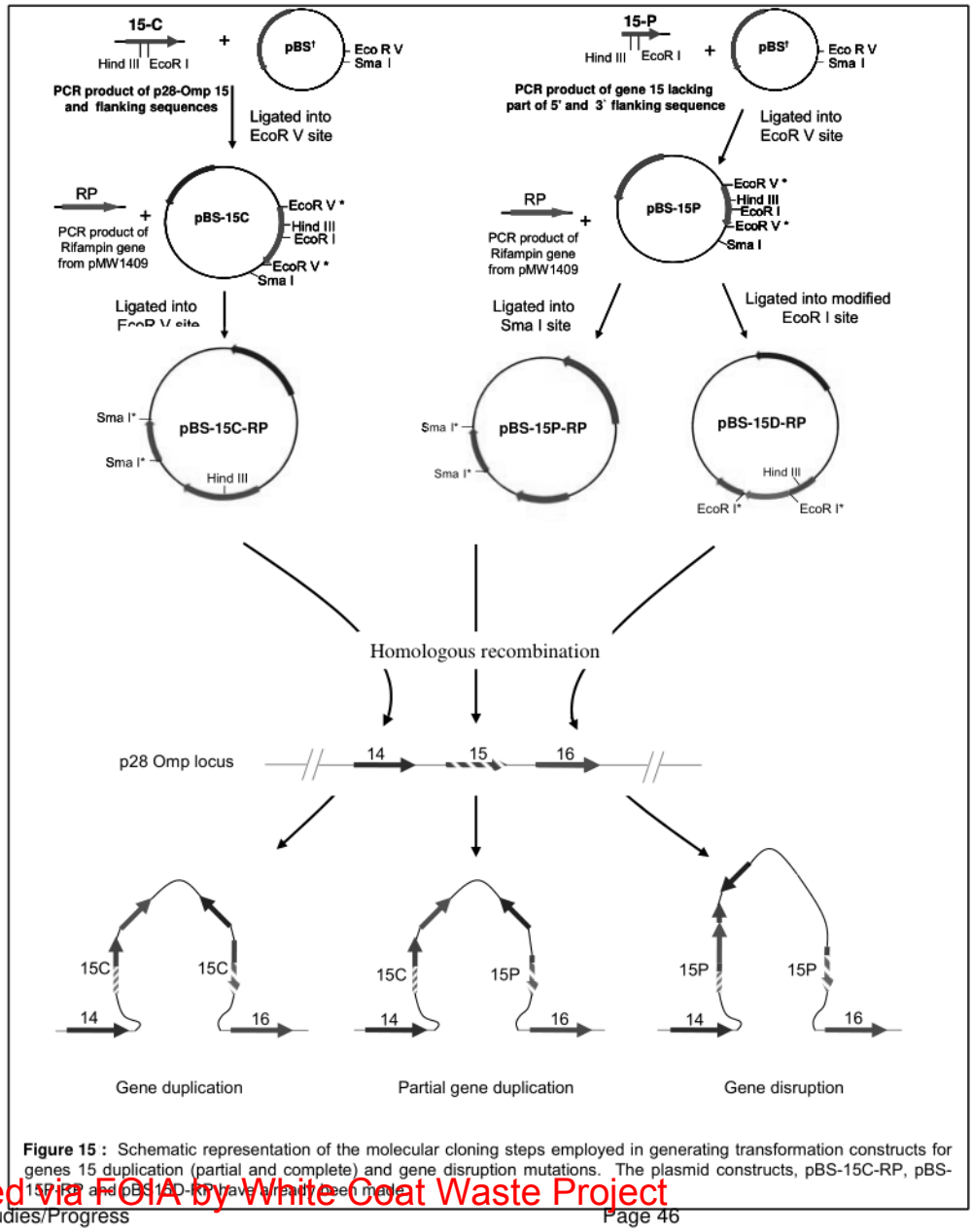

Figure 15 : Schematic representation of the molecular cloning steps employed in generating transformation constructs for genes 15 duplication (partial and complete) and gene disruption mutations. The plasmid constructs, pBS-15C-RP, pBS-15P-RP and pBS-15D-RP have already been made.

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pBS-15C-RP or pBS-15P-RP plasmids, respectively. The EcoR I site located in the middle of the coding sequence of gene 15 was linearized and filled in with T4 DNA polymerase to create blunt ends. This blunt ended plasmid was used to insert RP to generate the construct, pBS-15D-RP for use in gene disruption mutation experiment. All three plasmids linearized after digesting with Hind III (located in the ORF of gene 15) will be used in transformation experiments proposed in Specific Aim 4 (insertion mutations) (1). **Figure 15** summarizes the descriptions of various molecular manipulations employed to generate the three constructs and outlines the homologous recombination mutations expected to be developed in this study. The constructs that we designed are for use in insertion mutations to be achieved by homologous recombination (1,70) (as illustrated in the **Figure 15**). The pBS-15D-RP can also be used for the replacement mutation of gene 15 by homologous recombination (1). For this strategy, we will amplify the segment containing 5' and 3' end fragments of the gene 15 and the antibiotic resistance gene (regions spanning from 'red to green to red' fragment in the construct) and will be used in transformation experiments.

We performed two pilot transformation experiments using the plasmids pBS-15C-RP and pBS-15P-RP. Cell-free *E. chaffeensis* organisms obtained by differential centrifugation and resuspended in 0.25 M sucrose solution to prepare 10^8 bacteria per ml. Electroporation was performed at varying voltage settings ranging from1000 volts to 1900 volts using 10^7 cell-free *Ehrlichia* with or without 2 µg of pBS-15C-RP plasmid DNA. Transfer of plasmids into *E. chaffeensis* was assessed by Southern blot analysis of total DNA isolated and digested with Hind III restriction enzyme and probed with a ^{32}p labeled probes containing pBS-15C-RP and *E. chaffeensis* 16 S rDNA segments. The plasmid DNA was detected in DNA derived from electroporated organisms, but not in non-electroporated organisms (not shown). Subsequently, 1800 volts was selected for the pilot transformation experiments using pBS-15C-RP and pBS-15P-RP constructs. After electroporation, bacteria were transferred to culture flasks containing uninfected macrophage cultures in 5 ml of media and incubated at 37°C. After 24 h, the media was supplemented with rifampin. About 0.5 ml of the culture was

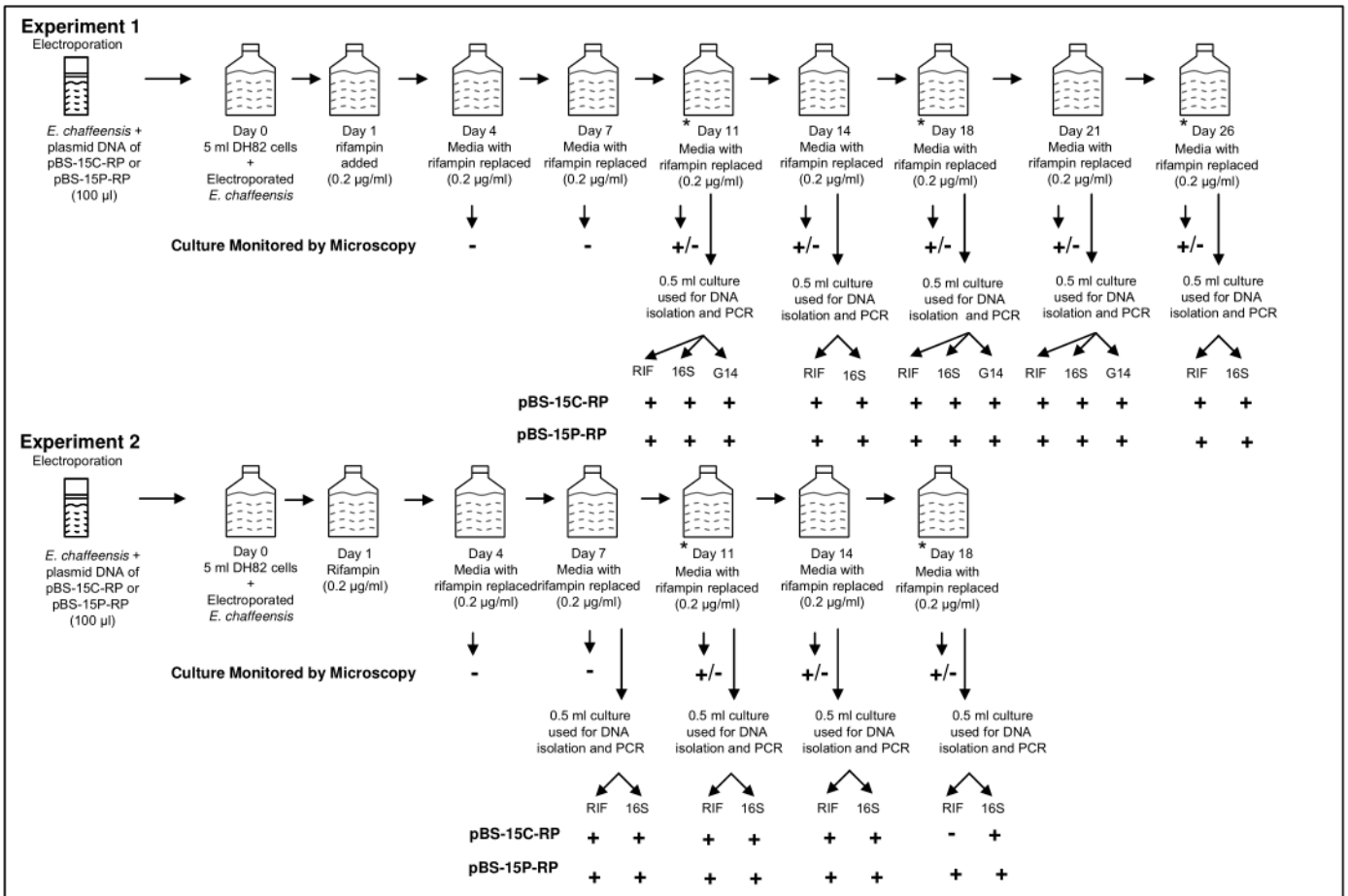

Figure 16: *E. chaffeensis* transformation experiments. Experiments 1and 2 included controls having the plasmid DNA with no electroporation or electroporation without a plasmid. Uninfected DH82 macrophage cultures were also maintained. All these cultures tested negative for *E. chaffeensis* when cultivated in the presence of rifampin. A positive control (electroporation with out a plasmid) and in the absence of rifampin also was included in each experiment. These control flasks yielded *E. chaffeensis* positives within seven days. (Addition of rifampin caused the *E. chaffeenisis* in DH82 culture flasks to disappear with in 4 days). * During the media change, DH82 cells were added to maintain the confluence of the flasks; the symbols - and +/- for culture monitoring refers to the absence or presence of morulae like structures in transformed cultures; while + or – for PCR results refer to positive or negative in a PCR assay (PCR assays were performed for 16S rRNA or p28-Omp gene 14 or rifampin resistence genes).

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harvested at every 3-4 days post infection beginning from day 4 and was used to monitor the *Ehrlichia* growth by microscopy and PCR analysis. Microscopic examination of the cultures from the first experiment revealed morulae like structures from day 11 to 26 in flasks containing cultures that were electroporated with pBS-15C-RP or pBS-15P-RP. Similar results were also noted in the second experiment, with morulae-like structures observed starting from day 7 (**Figure 16**). DNA isolated from all samples was analyzed by PCR for the rifampin resistance gene and by TaqMan-based real time PCR for 16S rDNA of *E. chaffeensis* (68,91). The transformation experiments included multiple controls: 1) electroporation of *E. chaffeensis* without a plasmid DNA; 2) non-electroporated *E. chaffeensis* cultures containing plasmid DNA cultured in the presence of rifampin; 3) as in control 2 but the rifampin was not added to the culture flasks; 4) uninfected DH82 cultures were grown to serve as a negative control. Rifampin resistant *E. chaffeensis* was not detected in flasks from controls 1, 2 and 4. *E. chaffeensis* was detected from day 7 in the flasks for control 3. We are now repeating these experiments and efforts are underway to determine if the electroporated DNA in the genome.

One of the challenges in using rifampin is the possibility of generating spontaneous resistance resulting from a single amino acid change in the RNA polymerase gene (71). Therefore, we evaluated additional antibiotics that inhibit the growth of *E. chaffeensis*. Our studies revealed that 10 µg/ml of chloramphenicol, 80 µg/ml of gentamicin and 50 µg/ml of spectinomycin are effective in inhibiting *E. chaffeensis* growth completely. The chloramphenicol acetyl transferase and gentamicin resistance genes are available from plasmid vectors, such as the Gateway pDEST^{TM} 10 vector (Invitrogen, Carlsbad, CA). A construct containing a spectinomycin resistance gene has been described recently (30). We obtained this construct from Dr. Uli Munderloh (University of Minnesota, St. Paul, MN). The antibiotic resistance genes were already engineered into the pBS-15D constructs by compiling strategies similar to those described above. In addition, we replaced *rpsL* promoter of *R. prowazekii* with the promoter of the *E. chaffeensis* transcription regulator (TR) gene. The TR gene is located upstream of the p28-Omp locus (42,50) and our microarray analysis revealed that the TR gene is not expressed in both tick cell and macrophage derived *E. chaffeensis*. The TR promoter containing constructs were selected using the appropriate antibiotics which verified that the promoter was active in *E. coli*. These additional constructs will be useful to serve as alternative approaches to introduce mutations in *E. chaffeensis* genome either by insertion or replacement mutations (1).

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RESEARCH DESIGN AND METHODS:

Specific Aim 1: Establish *in vitro* transcription system for mapping *Ehrlichia* promoters.

Importance: *E. chaffeensis,* primarily an intracellular obligate pathogen, must maintain a persistent infection in both tick and vertebrate hosts to successfully complete its life cycle. We presented several lines of evidence for host cell-specific protein expression in *E. chaffeensis.* The differentially expressed proteins included from the p28-Omp locus. The pathogen primarily expresses p28-Omp 19 gene product in a macrophage environment, whereas in tick cells the expressed protein is the product of p28-Omp 14 gene. We also presented evidence that the host cell specific differential expression is resulting from altered levels of transcription. Currently, there are no methods described in the literature that aid in the transfer of genetic material into *E. chaffeensis.* In the absence of a genetic transformation system, characterizing the gene function or the regulation of gene expression is challenging. Transcription analysis *in vitro* can serve as an excellent complementary tool for understanding the regulation of gene expression. For example, an *in vitro* transcription system has been developed for *C. trachomatis* that has been extensively used to map the regulation of gene expression (85,95,102). Regulation of gene expression is a fundamental process that controls protein synthesis and alterations in protein expression in *Ehrlichia* in response to vertebrate and invertebrate host systems. Thus, the establishment of a method to study transcription *in vitro* will have broader implications. For example, the methods will help assess how the tick-transmitted pathogens evade host clearance and aid in the identification of targets for controlling *Ehrlichia* infection. Quantitative assays by real-time RT-PCR and Western blot methods will be established in this specific aim for mapping variations in the *Ehrlichia* gene transcription using *in vitro* transcription system.

Experiment 1: *In vitro* transcription and translation system: In our preliminary studies, we already demonstrated a method for *in vitro* transcription coupled with translation using crude extracts of *E. chaffeensis*. We also showed that *E. coli* RNA polymerase can serve as a surrogate system to drive expression from *E. chaffeensis* promoters. The assays were extended for 90 min to get maximum expression. However, to measure the differences in the rates of transcription, it is important to identify conditions where the reaction is in a linear phase. Therefore in the first set of experiments, we will perform the following 1) *in vitro* transcription and translation for various time points after adding the *E. chaffeensis* lysates; 2) experiments to measure the rates of transcription and translation using varying concentrations of *E. chaffeensis* lysate and 3) experiments with lysates prepared from *E. chaffeensis* grown in macrophage and tick cells. ***The experimental control will include a non-infected DH82 or tick cell lysate control***. The reactions at different time points will be stopped by adding a stop solution containing 50 mM EDTA and using heat inactivation at 80° C for 15 minutes. Transcription will be quantitated by real time RT-PCR and confirmed by Northern blot or primer extension methods. Rates of translation relative to transcription will be measured by quantitating the protein synthesis from a marker protein and by comparing the results with RNA data. Based on our analysis from *E. chaffeensis* RNA that showed differences in transcription in genes 14 and 19, we expect to see variations in the rates of transcription for promoter constructs for these two genes. The *in vitro* assays are critical for examining if there are differences in the rates of transcription and translation.

Real time RT-PCR to map the transcription *in vitro*: We have considerable experience in developing real time RT-PCR assays (91,92). Primers and TaqMan probes will be designed to amplify a segment of the reporter gene transcript made from reporter plasmids. Initial standardization of the real time PCR assays will be performed using pPROBE-NT plasmid as the template for a GFP reporter gene. Subsequently, their value in detecting GFP transcripts will be established with RNA isolated from *E. coli* cultures that express the GFP protein from pPROBE-NT14 or 19 constructs.

Northern blotting: To confirm the transcription levels in the *in vitro* transcription experiments, Northern blot will be performed essentially as in the preliminary studies described above, but using probes specific for GFP gene transcripts. The GFP specific Northern blot probes will be generated from the GFP coding sequence by PCR.

Western Blot assay to measure GFP expression: Polyclonal sera against GFP (Rockland Immunochemicals, Gilbertsville, PA) will be used to determine the GFP synthesis in the *in vitro* transcription coupled translation assay. Densitometry analysis will be performed to measure the GFP protein detected by Western blot. We

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have already used the method for performing Western blot analysis in our preliminary studies. Comparison of RNA data with GFP protein synthesis will allow us to measure variations in the rates of transcription and translation.

The comprehensive analysis of these experiments will help us to establish the protocols for mapping quantitative differences in the rates of transcription and translation. As the *in vitro* transcription coupled translation uses all the components of *Ehrlichia*, we expect to see the differences in the rates of transcription to that of translation. The experiments will also be performed using *E. coli* transcription coupled translation system. The *E. coli* system is designed to provide the maximum rate of transcription and translation. Thus it will serve as a good control for mapping differences in the transcription and translation of *Ehrlichia*. Likewise, transcripts made from GFP constructs with *E. coli* extracts will allow us to see whether there are any differences in the RNA polymerase activities of *E. chaffeensis* to that of *E. coli.*

Experiment 2: Obtain preparations of *Ehrlichia* RNA polymerase complex to use in the *in vitro* system:
We are currently using crude extracts of *Ehrlichia* RNA polymerase. In this experiment, we will attempt to purify *E. chaffeensis* RNA polymerase. ***One of the challenges using a crude extract is that the extract may contain regulators of gene expression that may interfere with our subsequent studies proposed in specific aim 3***. Therefore, we propose to purify *E. chaffeensis* RNA polymerase complex. Purification protocols for RNA polymerase have been well established in literature for isolating active enzymes from many bacterial species (15,96). The methods are fairly simple and straight forward. We are encouraged by the fact that a similar heparin column-based purification protocol for *C. trachomatis* has been described recently (15). *Ehrlichia* species resemble in many ways to *Chlamydia*, such as their intracellular location and the morphological structure of having dense core and reticulate cells (3,45,67). Thus, we believe that the protocol described for *Chlamydia* will be useful in purifying *E. chaffeensis* RNA polymerase. We will use the following RNA polymerase purification method.

The heparin-agarose purification method described by Chamberlin *et al.* (15) will be modified for *E. chaffeensis* use. Briefly, *E. chaffeensis* will be grown in macrophages or tick cell line by following the standard culture protocols. A large volume of cultures (up to 200 ml or more) will be collected when infectivity reaches nearly 80% and particularly when *E. chaffeensis* is in reticulate form as this form represents the metabolically active state. Cultures will be harvested by centrifuging at 16,000 g for 15 min at 4°C. (All subsequent steps will also be performed at 4°C.) The culture pellet will be resuspended in 20 ml of phosphate-buffered saline (pH 7.5) and host cells will be lysed by sonication. The lysate will be centrifuged at 3,000 g for 10 min, and the supernatant will be transferred into a clean tube, passed through 10µ, 5µ and 3µ filters. The filtrate containing *E. chaffeensis* will be centrifuged at 16,000 *g* for 10 min. This *Ehrlichia* pellet will be resuspended in 2 ml of freshly prepared lysis buffer [10 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 1 mM EDTA, 7.5% (v/v) glycerol, 0.3 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride, 150 mg of lysozyme per ml, 10 mg of pepstatin A per ml containing 0.6 M NaCl]. Twenty microliters of 10% (v/v) Nonidet P-40 (Sigma Aldrich, St. Louis, MO) will be added, and the mixture will be incubated on ice for 5 min. The *E. chaffeensis* organisms will be lysed by sonication. The lysate will be centrifuged at 16,000 *g* for 30 min, and the supernatant fluid will be collected and centrifuged two additional times for 15 min each. The supernatant will be diluted with 1.4 volumes of buffer containing 10 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 1 mM EDTA, 7.5% (v/v) glycerol, 0.3 mM DTT to give a final concentration of 0.25 M NaCl. This diluted supernatant fluid will be mixed for 1 h with 1 ml of heparin-agarose (Sigma Aldrich, St. Louis, MO) previously equilibrated with buffer containing 0.25 M NaCl. The heparin-agarose column will be packed and washed by gravity flow with 20 ml of buffer containing 0.25 M NaCl. The *E. chaffeensis* RNA polymerase from the column will be eluted with buffer containing 0.6 M NaCl and 0.5 ml fractions will be collected. Fractions will be dialyzed against storage buffer [10 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 0.1 mM EDTA, 0.1 mM DTT, 100 mM NaCl, 30% (v/v) glycerol] and stored at -70°C. Peak fractions will be identified by their *in vitro* transcription activity.

Potential pitfalls and alternative strategies: The establishment of real time RT-PCR assay is straight forward and we do not anticipate problems in standardizing this technique. Northern blot and primer extension experiments will be performed as in preliminary studies but using probes specific for the proposed experiment. Western blot assay to measure the GFP expression is also standardized in our preliminary studies. We expect that the proposed RNA polymerase isolation method will allow us to recover relatively pure and active enzyme. However, we may encounter problems recovering a sufficient amount of enzyme for performing the *in vitro* assays. We will attempt to overcome this limitation by growing cultures in higher volume. We believe

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that this will be best achieved by growing several flasks simultaneously and pooling cultures prior to isolating RNA polymerase. Another possible limitation will be that *E. chaffeensis* RNA polymerase has lower affinity to heparin agarose column. We will modify salt concentrations to identify appropriate binding conditions to overcome this limitation. The PI's (Ganta) basic training in biochemistry including a great deal of enzyme purification and characterization experience will be useful for troubleshooting problems and overcoming any challenges of enzyme purification. In the unlikely event that we do not get sufficient enzyme by this purification method, we will attempt to identify the gene of *E. chaffeensis* RNA polymerase from the currently available genome sequence (42) and use the information to clone and express the protein in *E. coli*. Standard protocols of molecular cloning and expression methods will be used for the recombinant RNA polymerase expression. Our laboratory has expertise in expressing proteins in *E. coli* (19,73,74). We have also demonstrated that *E. coli* RNA polymerase can serve as the surrogate system to drive expression from *E. chaffeensis* promoters. Thus, in the very unlikely event that both our efforts to isolate purified *E. chaffeensis* RNA polymerase fail, we will use *E. coli* RNA polymerase to drive the *in vitro* transcription. *E. coli* RNA polymerase has been extensively used for studies on *C. trachomatis* to advance understanding of the gene expression for several genes (57,96,97,101). So, we expect to obtain meaningful data regarding the regulation of gene expression from *E. chaffeensis* genes 14 and 19 of the p28-Omp locus independent of whether or not we can purify RNA polymerase.

Specific Aim 2: Map transcriptional machinery in differentially expressed genes of the p28-Omp locus.

Importance: In prokaryotes, control of transcriptional initiation is the primary site for control of gene expression (60). Typically, transcription initiation is controlled by two DNA sequence elements that are upstream of the site of transcription initiation and referred to as the -35 and -10 positions. These sequence elements are regarded as the promoter sequences. The activity of RNA polymerase at a given promoter is regulated by interaction with accessory proteins, referred to as the regulatory proteins, which affect the ability of RNA polymerase to recognize the transcription start sites. These regulatory proteins can act both positively to serve as the activators and negatively to serve as the repressors. The accessibility of promoter regions of prokaryotic DNA is mostly regulated by the interaction of proteins with sequences near the RNA polymerase binding sites. The regulator regions adjacent to the promoter elements play an important role in altering the gene expression. We hypothesize that the altered rates of transcription in *E. chaffeensis* are the result of the control of gene expression. It is entirely unknown about how this important tick-transmitted rickettsia controls its gene expression to support its growth in macrophages and tick cells. Basic knowledge of the regulation of gene expression must be understood. The experiments proposed in this specific aim address this important question.

Experiment 1: Primer extension analysis to confirm the transcription start sites: In our preliminary studies, we mapped the transcription start sites for genes 14 and 19 of the p28-Omp locus by primer extension and RNase protection assays. In this experiment, we will design a primer that can bind to the GFP transcript near the 5' end to be used for mapping transcription start sites using *in vitro* generated transcripts. ***The purpose of this experiment is to verify that the RNA polymerase recognizes the same promoter regions as those observed for the transcripts of gene 14 and 19 in E. chaffeensis***. Two forms of RNA will be prepared by using either *E. coli* RNA polymerase or *E. chaffeensis* RNA polymerase complex in the in vitro transcription assay. Then, about 10 µg of *in vitro* generated RNA from the genes 14 and 19 constructs will be used to perform primer extension analysis. Complementary primers designed to bind GFP will be used in the experiment after it is end labeled with γ-^{32}p ATP using T4 polynucleotide kinase (78). The primer extended products will be resolved on a denatured 6% polyacrylamide sequencing gel, and the presence of radiolabelled primer extended products will be detected by exposing the resolved gel to an X-ray film. These protocols are very similar to the primer extension analysis performed and reported in the Preliminary Studies section. In this experiment, we expect to observe that the transcription start sites are identical to those observed in RNA isolated from *E. chaffeensis*. Alternatively, we may see the transcripts generated in the *in vitro* transcription system have different initiation sites of transcription. Although this is not an anticipated result, this discovery could offer exciting new possibilities for examining alternative transcription start sites in *E. chaffeensis*. If such results are observed, we will attempt to modify experimental conditions to promote the expression from the correct transcription start sites in the *in vitro* transcription system. The modifications to the experiment may include altering the temperature and/or salt concentrations during the *in vitro* transcription assays.

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Experiment 2: Sequence deletion analysis to identify the RNA polymerase binding site: The goal of this experiment is to identify the minimal core promoter required for the RNA polymerase to drive the transcription for genes 14 and 19. Many protocols are described in the literature for performing deletion analysis (95). To generate deletions from 5' end, PCRs will be performed using different 5' end primers with the same 3' end primer used in constructing the genes 14 and 19 promoter constructs (as described in the preliminary studies). These PCR-based deletion analyses also will be performed to shorten sequence from 3' end. We already performed pilot studies using ~60 base pair deletions in our preliminary studies. Similar approaches will be employed in this study for fine mapping the promoter regions. We will perform initial screening of all deletion constructs by transforming into *E. coli*. The promoter activity in *E. coli* will be assessed by measuring the reporter gene GFP or β-galactosidase expression. When using GFP constructs, GFP expression will be monitored rapidly for GFP positive colonies. The GFP expression will also be confirmed by Western blot analysis on lysates prepared from *E. coli* colonies containing modified promoter constructs. Likewise, lacZ reporter gene activity will be assessed by measuring the β-galactosidase expression. Subsequently, the promoter activity and the rate of transcription will be assessed for the constructs using in vitro transcription coupled translation system for both *E. coli* and *E. chaffeensis* RNA polymerases. These deletion analyses will allow us to completely map the promoters required to drive the expression from the genes 14 and 19.

Experiment 3: The site specific mutations to identify the RNA polymerase binding sites: At the conclusion of the previous experiment, we will be able to identify the minimal sequences required to drive mRNA synthesis and determine all of the regions involved in gene regulation. The precise location of RNA polymerase binding sites can be mapped by site-specific mutational analysis. Various primers will be designed to introduce one or more mutations within the RNA polymerase binding region. The primers will be used in the PCR to amplify and clone to introduce mutations in the constructs. As proposed in the previous experiments, reporter gene activity will be assayed to map their impact on the RNA polymerase binding. Nucleotide modifications will impact RNA polymerase binding, thus leading to altered rate of transcription or to no transcription (86).

Potential pitfalls and alternative strategies: We are adept in performing all the proposed experiments. The *in vitro* analysis is important to verify the transcription start sites and ensure that the *in vitro* conditions do not influence the gene activity. In our preliminary studies, we have already performed primer extension experiments to map the transcription start sites of genes 14 and 19. Likewise, we have experience with PCR and cloning strategies for generating deletion and mutation analysis. In the event we encounter problems in generating sufficient deletions or mutations, we will look at alternative strategies. One alternative strategy is to generate nested deletions using Exo III and Mung Bean nucleases (40,72). The vectors we use have multiple cloning sites that aid in designing experiments for creating nested deletion analysis from either the 5' end or 3' end (62). In the past, we have used such a protocol to generate deletion mutations for mapping a human phosphofructokinase gene promoter and for generating nucleotide sequences of various DNA fragments (77). Promoter analysis by saturation mutagenesis can serve as a good alternative for aiding in our efforts to map the promoters (5). Our collaborator on this project, Redacted by agreement has considerable experience in mapping promoters by performing deletion and mutational analyses (37,97,109) (Appendix 5-7). His input will be sought in designing and executing these experiments. Together, our team has considerable expertise to conduct and troubleshoot the proposed experiments for this specific aim.

Specific Aim 3: Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19 of the p28-Omp locus:

Importance: The results from the experiments conducted in Specific Aim 2 will allow us to define unique regulatory machinery used by *E. chaffeensis*. This specific aim will address the biologically relevant question, how is *E. chaffeensis* able to regulate gene expression in response to its growth in vertebrate and tick cell environments? We hypothesize that *E. chaffeensis* alters its gene expression to overcome challenges that it may encounter in the dual hosts. This may be accomplished by the interactions of regulators such as enhancers and repressors with DNA elements upstream to the RNA polymerase binding sites. In this specific aim, we will prepare two cell lysates of *E. chaffeensis* grown in macrophages and tick cells. The lysates are expected to contain regulatory proteins that interact with the promoters of genes 14 and 19 of the p28-Omp locus. The experiments proposed in this specific aim are designed to identify unique differences in the regulators and regulatory elements that induce differences in the gene expression. Therefore, these

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```
Gene14-upstream  ttgctcaacccataaaataatggggaaattacctttctaggaagtttctcattatttaac
Gene19-upstream  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~tttt at
      Consensus  ------------------------------------------------------TTT-A-

Gene14-upstream  agttaactttctgtaaacttctaataacagtattttgttcactcttccccttaataaaaa
Gene19-upstream  tattgccacatgttaaaaataatctaaacttgtttttattattgctgcaggtaaataaaa
      Consensus  --TT--C------TAAA--T----TAA---T-TTTT--T-A-T--T-C---TAA-AAAA-

Gene14-upstream  tcataagtttacaataatgtcaaaaagatttctttttaaacacatttaaaatggctaaac
Gene19-upstream  ..atagtggcaaaagaatgtagcaataagaggggggggggggggactagtttataagtgc
      Consensus  --ATA-----A-AA-AATGT---AA--A------------------TA---T-------C

Gene14-upstream  cgttttctgctttattagaatgattcccaaataaattttaattaattactgttccgtatt
Gene19-upstream  tgtttttctcacctttacacatgatactatacttaaccagtttttttgctattacttacc
      Consensus  -GTTTT---C----TTA-A-----T-C-A-A---A------TT--TT-CT-TT-C-TA--

                                                  -35       AT            -10
Gene14-upstream  tattaatatatgttataatgtaattaaataaggatactagatttgctcataatgcatgta
Gene19-upstream  tgacgtaatatattaaattttccttacaaaagTTACCGatatttttatacaaaaattTATA
      Consensus  T------ATAT-TTA-A-T-T--TTA-A-AAG----C-A-ATTT--T---AA----T-TA

                                  *
Gene14-upstream  ctgaatttgtgatttgaaataacaagacttaaatgtcgaatttagcttctgtcctagtgg
Gene19-upstream  TTctgacttgcttttatatgacacttctactattgttaatttatttgtcactattaggtt
      Consensus  -T-----T----TTT--A--A----------A-TGT--A-TT-----TC--T--TAG---

Gene14-upstream  ataagtactttagcaagtggtaaaagcaagtctactcatatttttattaattaagtagta
Gene19-upstream  atat~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      Consensus  ATA------------------------------------------------------

Gene14-upstream  aagttaactatagattttattaaaatttttattctaatcactttaaatatcaattacttt
Gene19-upstream  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      Consensus  ------------------------------------------------------------

                                                    -35      AT
Gene14-upstream  tgttgtaaatttgaaagaaattttatattctagacTTGCTTttcttattatttctttcatTA
Gene19-upstream  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      Consensus  ------------------------------------------------------------

                 -10   *
Gene14-upstream  TTCTtaaatttttattatcttttataaaaggttattaaca
Gene19-upstream  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      Consensus  -------------------------------------------
```

Figure 17: Sequence alignment and the identification of putative promoters and various sequence elements in the sequences upstream from the protein coding sequences of *E. chaffeensis* p28-Omp genes 14 and 19. *, transcription start site mapped by primer extension and RNase protection anlysis; blue text, putative ribosome binding sites (RBS); red highlighted text, AT rich spacers; bold and yellow color highlighted upper case text, putative promoter regions -10 and -35; sea green, g-rich region in the gene 19 upstream sequence; pink underlined text connected with dotted line, inverted repeats; red underline text with connected dotted line, direct repeats. The g-rich region, inverted repeats, and direct repeats may represent DNA elements to which transcription regulators may bind.

```
            -35    AT-rich spacer     -10                                                    RBS
E.coli:     TTGACA     TTTA           TATAAT                                                 AGGAG
P28-14: ACTTGCTTTTC-TTTATTTCTTTCATTATTCTTAAATTTTTATTATCTTTTATAAA----------------------------AGGTT
P28-19: AGTTACCGATATTTTATACAAAAATTTATATTCTGACTTGCTTTTATATGACACTTCTACTATTGTTAATTTATTTGTCACTATTAGGTT
```

Figure 18: Comparison of putative promoters of genes p28-Omp 14 and 19 with the *E. coli* consensus promoter sequences. Transcription start sites for the genes were mapped by primer extension and RNase protection analysis and are identified in the figure with bold and gray color highlighted text. Putative -10 and -35 were shown in yellow color highlighted bold text and the putative ribosomal binding sites (RBS) were shown in blue and highlighted text. These sequences and their location upstream from the start sites were also presented in the Figure 1. *E. coli* AT rich spacer sequence is reported in (81). (Note: Dashes were introduced in the sequence of p28-Omp14 sequence to create alignment with the sequence of p28-Omp19)

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experiments are important to provide fundamental knowledge about strategies by which the tick transmitted intracellular rickettsiales evade the host clearance.

Experiment 1: Evaluation of DNA protein interactions**:** The goal of this experiment is to assess variations in the gene expression from genes 14 and 19 promoters under *in vitro* in the presence of *E. chaffeensis* lysate prepared from the cultures of bacteria grown in macrophages or tick cells. In this experiment, we will test the hypothesis that transcription from *E. chaffeensis* genes 14 and 19 differs because one or more regulators bind to DNA elements in the promoters and induce changes in the gene expression. *E. chaffeensis* may achieve these transcriptional changes due to differences in the binding of regulators (enhancers/suppressors). This exciting hypothesis will be tested by using *E. chaffeensis* extracts prepared from the cultures grown in two different host cell environments. *E. chaffeensis* lysates will be prepared as we described earlier (Appendix 4). Varying concentrations of the lysates will be added to reconstitute transcription. The rates of transcription will be measured. We expect to see lower levels of transcription for gene 14 and higher levels for gene 19 when macrophage derived *E. chaffeensis* extract is added. Opposite results are expected when tick cell-derived *E. chaffeensis* extracts are used. Controls in this experiment will include the addition of heat inactivated cell lysate or cell lysate alone (without *E. chaffeensis* RNA polymerase) or cell lysates prepared from macrophages or tick cells. The differences in transcription in the presence of lysates from *E. chaffeensis* grown in macrophages and tick cells will likely result from the binding of one or more regulatory proteins to the genes 14 and 19 promoters. Such binding of regulators and location of binding can be determined by gel mobility shift and DNase foot printing analysis described below.

We have identified significant differences in the putative promoter regions and in the sequences upstream from genes 14 and 19 (comparisons were provided in **Figures 17 and 18**). One obvious difference is that the length of the sequence is different between the gene 14 and gene 19 upstream sequences. Although the predicted -35 and -10 sequences are very similar to *E. coli* consensus sequences, they do differ at several nucleotide positions (**Figure 18**). AT rich spacers identical to *E. coli* were also detected. The predicted ribosome binding site (RBS) sequences are identical for both the genes 14 and 19, but differed slightly from the *E. coli* consensus sequence. Nucleotides at transcription start sites and relative locations of the transcription start sites are different for the genes 14 and 19. In addition, several unique sequences were identified in the regions upstream to the predicted -35 and -10 sequences for genes 14 and 19. They include a 'G' rich region unique to gene 19 and several direct and inverted repeats unique to genes 14 and 19. All or some of these unique sequences may serve as gene-specific DNA elements for binding of one or more regulators to control the transcription in a host cell-specific manner.

Experiment 2: Deletion and mutation analysis to verify the DNA protein interactions: The binding of regulatory proteins to the unique regions of genes 14 and 19 promoters will be assessed by gel mobility shift and DNase foot printing assays. The effect of the binding of regulators and transcription will be monitored by *in vitro* transcription assays. To confirm the specific binding of regulators and their effects on transcription, we will perform deletion and mutation analysis. Gel mobility shift assays also will be performed to further confirm the loss of DNA binding of regulatory proteins in constructs lacking DNA elements.

Gel Mobility shift assay: Gel mobility shift assay, also known as electrophoretic mobility shift assay, is a rapid and simple technique for characterizing DNA-protein interactions. It is based on the differential mobility of DNA before and after binding to protein(s) in a non denaturing polyacrylamide or agarose gels (56). We will use a commercial kit to perform non-radioactive gel shift assay (Roche Applied Science, Indianapolis, IN). The non-radioactive gel mobility shift assay involves labeling the 3' end of a double-stranded DNA fragment with terminal transferase and DIG-ddUTP1, by incubation DIG-labeled DNA with cell extract for 15 min and resolving the mixture in a 12.5% non-denaturing polyacrylamide gel. The resolved DNA and DNA protein complexes will be transferred to a nylon membrane to detect DIG-labeled DNA-protein complexes with alkaline phosphatase-conjugated anti-DIG antibody and CSPD chemiluminescent alkaline phosphatase substrate. Chemiluminescent signals will then be recorded by exposing an X-ray film. The *E. chaffeensis* protein extracts for gel mobility shift assay will be prepared as we originally described in (74). To determine the specific interactions between regulators and DNA elements, gel mobility shift experiments will include controls that contain cold (unlabelled) competitor DNA segments or sheared salmon sperm DNA as a non-competitor. We expect that the addition of cold DNA will compete for interactions with the specific sequence regulators. Similarly, non-specific DNA control will aid in further demonstrating specific interactions.

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DNA footprinting: DNA foot printing is a method for determining the exact DNA sequence to which a particular DNA binding protein binds (33). The DNA and protein interactions will be performed as described above for gel mobility shift assay except that the DNA will be end-labeled with γ-P^{32} ATP at the 5' end usingT4 polynucleotide kinase. This technique is a simple conjoining of the Maxam-Gilbert DNA-sequencing method and the technique of DNase-protected fragment isolation (33). The ^{32}p labeled DNA will be partially degraded by DNase in the presence and absence of *E. chaffeensis* lysate and visualized by electrophoresis and autoradiography. DNase treated DNA in the absence of a lysate will serve as the control to identify protein binding sites referred to as protective "Foot print" of binding protein. DNA footprinting assay is a fairly standard protocol and several commercial vendors sell kits to perform DNase foot-printing experiments. We will use a commercial kit sold by Amersham Pharmacia Biotech to perform this analysis (SureTrac Footprinting Kit).

Determine the gene regulators: Gene-specific transcription regulators involved in the host-specific differential regulation will be identified with the aid of mass-spectrometry analysis. Proteins bound to specific DNA regions will be identified with the aid of the gel mobility shift experiments (described above). Subsequently, bound proteins from the acrylamide gels resolved by gel mobility shift assays will be recovered and subjected to trypsin digestion and LC-MS/MS analysis will be performed to establish the identity of those proteins. We have performed numerous LC-MS/MS analysis experiments during the last 4 years (89,90) and this experience will be valuable in accomplishing these tasks.

Potential pitfalls, alternative strategies and expected outcomes: The experiments in this aim involve the preparation of *E. chaffeensis* lysates, gel mobility shift, DNase foot printing assays, deletion/mutational analysis and LC-MS/MS analysis. We have previously used all of the protocols and also have demonstrated our expertise in performing mobility shift assays (74) (Appendix 4). The reagents for deletion/mutational analysis will be mostly the same as those to be prepared in specific aim 2. Potential pitfalls and alternative strategies are already described under that aim. DNase foot printing will be performed using a commercial kit. This is one experiment that has never been performed in our laboratory. However, we believe that this will not be a limitation because of the following reasons. The assay will be performed using a commercial kit. Second, our research group has been active in performing numerous molecular biology experiments for quite some time. Finally, our collaborator also has the expertise in this area of research. Thus, we are optimistic that we can troubleshoot any potential problems that may arise in executing this experiment. We have considerable expertise to perform mass spectrometry analysis and thus we do not anticipate problems executing these experiments. The experiments proposed in this aim will allow us to directly test our hypothesis to map unique host-specific differences in gene regulation and regulatory proteins that contribute to the differential regulation. Establishing the identity of gene regulators will open many new opportunities to understand the differential gene regulation by *Ehrlichia* species in a host cell-specific manner. This study will ultimately lead to the identification of unique targets to control *E. chaffeensis* infection in people.

Specific Aim 4: Evaluation of knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific expression. In this aim, we will test the hypothesis that unique host cell-specific expression sites from the p28-Omp locus are essential for the pathogen's survival in vertebrate and tick cell environments.

Importance: Much of the research on this organism is hampered due to the lack of genetic manipulation systems. The availability of a genetic manipulation system will significantly aid in mapping the genes critical for *E. chaffeensis* survival and pathogenicity. Because *E. chaffeensis* is a Gram-negative organism, existing methods of introducing DNA into other Gram-negative bacteria, such as *E. coli* and *Mycoplasma* species should be effective (55,56). Because *E. chaffeensis* is an obligate intracellular pathogen, the transformation of foreign DNA into *E. chaffeensis* appears complicated. However, this may not be a serious limitation as several recent reports on *Rickettsia* species*; R. prowazekii, R. typhi* and, *R. monacensis*, three closely related obligate intra-cellular bacterial to *E. chaffeensis* demonstrated successful transformation (69,71,104). Transformation in *Rickettsia* species is reported using a green fluorescence protein and genes that confer resistance to rifampin, chloramphenicol and erythromycin (69,71,104). Importantly, non-rickettsial genes are also used to transform rickettsiales. Recently, Long *et al.*, presented evidence for the transformation of *E. muris*, a species closely related to *E. chaffeensis* (51). In this study, the authors transformed *E. muris* with a plasmid containing *E. chaffeensis* p28-Omp gene 19 promoter tagged in front of a chloramphenicol acetyl transferase gene. The results are very encouraging and suggest that transformation is a possible task for *Ehrlichia* species.

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Moreover, transformation using transposon-based mutations has also been demonstrated for *Rickettsia* and *Anaplasma* species (4,43,68). The need to establish a genetic transformation system for *E. chaffeensis* and the availability of protocols for transformation used for several other rickettsiales provide considerable justification to undertake the proposed experiments in this new specific aim.

We presented several lines of evidence for the unique expression sites from the p28-Omp locus of *E. chaffeensis.* The pathogen in the macrophage environment predominantly expresses p28-Omp 19. In tick cells, *E. chaffeensis* expresses the p28-Omp 14 gene product. We believe that the differential expression from the p28-Omp multigene locus is critical for the pathogen's adaptation and survival in the vertebrate and tick cells. The demonstration of unique protein expression from the p28-Omp gene locus in vertebrate host and tick cell is similar to the *B. burgdorferi* OspA and OspC expression in tick and vertebrate hosts, respectively (31,66). Recent studies on *B. burgdorferi* suggest that disruption of the genes encoding these proteins results in the pathogen's failure to colonize in tick or vertebrate hosts (66). In this Specific Aim, we propose experiments to develop transformation and mutation analysis systems to evaluate the impact of the disruption of genes 14 and 19 on *E. chaffeensis* growth in macrophages and tick cells.

Experiment 1) P28-Omp 15 gene mutational analysis: For initial experiments, we selected the gene 15 of the p28-Omp multigene locus for mutational analysis. This gene was chosen as it is transcriptionally silent or expressed at low levels in *E. chaffeensis* grown in macrophage or tick cells. In the first set of experiments, we will use plasmid constructs which will allow complete or partial gene 15 duplications or gene 15 disruption by insertion mutations. In the preliminary studies, we already prepared plasmid constructs needed for completing these experiments. The initial designing included rifampin resistance gene to serve as the marker to select transformants using rifampin. As the rifampin resistant *E. chaffeensis* can be developed easily as the result of a single amino acid change in the RNA polymerase (71), we also identified alternative selection markers to confer resistance to, spectinomycin, gentamycin or chloramphenicol. None of these antibiotics are choice of drugs to control Ehrlichial infections. Thus, they are suitable to use for the transformation studies. The spectinomycin, chloramphenicol acetyl transferase and gentamicin resistant genes are already inserted into the plasmid constructs (preliminary data).

The transformation protocol will be followed essentially as described by our collaborator, Dr. Redact Redact (68) with some minor modifications as described in preliminary studies. The presence of genetically-modified *E. chaffeensis* will be assessed in cultures grown in the presence of an antibiotic by examining culture viability, genomic DNA analysis by Southern blot and PCR. PCRs will be performed using primers spacing the expected insertion regions or with primers targeting a drug resistance gene or a combination of the two. Modifications may be made to some of the protocols used to optimize conditions for transformation.

Potential outcomes, pitfalls and alternative strategies: We anticipate three outcomes for this experiment: 1) there will be no change in the growth of *E. chaffeensis* in all three transformations (partial gene insertion, gene duplication or complete gene disruption). This result will indicate that duplication or disruption does not harm the growth of *E. chaffeensis*. This result will be encouraging and will allow us to proceed immediately with mutational analysis for p28-Omp genes 14 and 19; 2) There will be no change in the growth or morphology of *E. chaffeensis* in gene duplication or insertion of a partial gene 15, but disruption will result to a lack of growth or retarded development of *E. chaffeensis* either in macrophages, tick cells or in both cell backgrounds. This outcome is also encouraging and may suggest that although we detected low levels of the p28-Omp gene 15 transcripts in *E. chaffeensis,* its disruption may impact the pathogen's survival or the regulation of gene expression for transcriptionally active genes in its surrounding region. If the growth of *E. chaffeensis* is retarded only in macrophages, we will reevaluate its growth in the tick cell culture to examine if the growth kinetics will return to normal levels; 3) the transformation will not result in antibiotic resistant *E. chaffeensis.* This possibility cannot be ruled out as a result of the inability of *E. chaffeensis* to support the expression of antibiotic resistance protein using the *rpsL* promoter of the *R. prowazakii*. To overcome this challenge, we have already engineered a transcription regulator (TR) promoter of *E. chaffeensis* into the constructs (preliminary data), which will be used in the transformation experiments. We believe that the contributions of our collaborator, Dr. Wood will be very valuable to further troubleshoot the problems we may encounter during the transformation experiments.

Experiment 2) Mutations spanning the genomic region of p28-Omp 14 or 19 genes: The recombinant plasmids for transformation needed to cause disruption or duplications (complete or partial) of genes 14 or 19

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will be prepared using a strategy similar to that outlined in the preliminary studies for gene 15. The transformation plasmids for mutational analysis in the genomic regions spanning these two genes will be used similarly in homologous recombination experiments. The growth of *E. chaffeensis* will be assessed in both macrophage and tick cell lines with selection for resistance to antibiotics used. The transformants will be carefully analyzed to identify mutants in macrophage and tick cells using molecular and proteomic tools (references). Mutations in *E. chaffeensis* will be evaluated by Southern blot and by PCR analysis of genomic DNA. Whole genome microarray analysis (as described in preliminary data) will also be performed to help assess the impact of these gene disruptions or duplications on the global changes in the pathogen gene expression.

Potential outcomes, pitfalls and alternative strategies: The disruption of gene 14 may lead to the inability of *E. chaffeensis* to grow in tick cells, but may not result in any change to the pathogen in the macrophage cell line. Similarly, the deletion of gene 19 may lead to failed or retarded growth in macrophage cell lines, while apparently causing no effect in the tick cell line. These results will support our working hypothesis that gene 14 and gene 19 are critical for the pathogen's survival in tick cell and macrophage environments, respectively. It is possible that the disruption of these two genes may have no impact on the growth of *E. chaffeensis* either in invertebrate or vertebrate cell lines. These results could indicate that the disruptions are overcome by the expression of one or more genes from the other regions of p28-Omp multigene locus. It is also possible that no other gene expression may be complemented for the loss of these two genes. To explore these two possibilities, RNA will be isolated from these two *E. chaffeensis* mutants and tested for the presence of transcription by microarray analysis spanning all the 22 genes of the p28-Omp locus. RNA analysis will also be performed by Northern blot or real-time RT-PCR analysis to examine the presence or absence of transcripts for genes 14 and 19. Using microarray analysis, we will also evaluate whether the mutations have any impact on the global changes of gene expression. In addition, proteomic analysis will be performed to determine differences in the protein expression as we described earlier (89). The gene duplication or partial gene insertions of genes 14 and 19 may have no impact on the pathogens growth, or possibly over-expression might result in better growth or slightly altered growth kinetics of *E. chaffeensis*. All of these possibilities will be carefully explored.

At the completion of the experiments proposed in this new specific aim, we expect to 1) establish genetic transformation and mutation protocols; 2) define the importance of differential expression from the p28-Omp locus for the pathogens survival in macrophage and tick cell environments; and 3) assess whether *E. chaffeensis* has a mechanism to overcome the loss of a gene by inducing expression from another expression site. We are optimistic that these exciting new experiments will be completed with no potential challenges. Together with our collaborator, Dr. Woods' expertise on genetic transformation system, we are well-prepared to troubleshoot the experimental challenges. This is further strengthened by the fact that more and more evidence has presented for genetic transformation systems in *Rickettsia, Anaplasma,* and *Ehrlichia* species in recent years. Therefore, we believe that the experiments proposed in this specific aim will allow us to address very important biologically relevant questions about the significance of host-specific antigen expression in *E. chaffeensis*. This study will also open new directions in this field of research and explore relevant issues about the infection *in vivo*, such as evaluating the impact of disruption of protein expression from one or more genes on infection, persistence and pathogenicity.

SUMMARY: In this study, we proposed novel experiments to map the molecular basis for differential gene expression and to asses the impact of the loss of uniquely expressed genes for the pathogen's survival. At the conclusion of the proposed project, we will gain considerable knowledge about how tick-transmitted pathogens, *E. chaffeensis* in particular, will respond to the host environment. The study will have much broader implications for understanding the molecular mechanisms, tick-transmitted pathogens employ to survive in vertebrate and invertebrate hosts.

TIME FRAME: We are already making progress on all four specific aims. Therefore, our studies will have already been initiated and will be ongoing in year 1 of the project. However, for the ease of overall handling of the project, the major emphasis in years 1 and 2 will be on Specific Aim 1 and 2. In year 3 and continuing through year 4, the majority of the work will be focused on Specific Aim 2 and 3. The work proposed in Specific Aim 4 will be completed in years 4 and 5.

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March 14, 2007

Dr. Roman Ganta
Department of Diagnostic Medicine / Pathobiology
College of Veterinary Medicine
Kansas State University
Manhattan, KS 66506

Dear Roman:

I am writing in support of your proposal entitled "Regulation of Gene Expression in *Ehrlichia chaffeensis*", for which I would be pleased to serve as a collaborator and consultant. As you know, I have been involved in the molecular genetic characterization of bacterial pathogens for the past thirty years and I have considerable expertise in the manipulation of bacteria for which there are few genetic tools available. The combination of your expertise with this bacterium and any contribution I may make will help ensure the success of this project.

Please feel free to consult me as needed for any issues that arise related to the proposed experimental plan. I am willing to collaborate on this project as needed throughout its duration. Our laboratories are Redacted by apart and it should not be a problem to arrange face to face discussions as needed.

I have enjoyed our discussion of science during the time I was at Kansas State University and I hope that we will have many more opportunities to work together.

Sincerely,

Personal Info

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Redacted by agreement

March 8, 2007

Dr. Roman R. Ganta
Department of Diagnostic Medicine and Pathobiology
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas 66506

Dear Roman,

It was a pleasure having the opportunity to speak with you again about your proposed studies. I am very impressed by your progress since we discussed your project at the American Society for Rickettsiology meeting in Asilomar last September. Your approach to understanding the differential expression of the ehrlichial multigene p28 locus is a good one and your identification of loci that are highly expressed and others that are not expressed raises some intriguing questions. Understanding how differential gene expression affects intracellular growth is the next barrier that must be overcome and I am glad you have decided to address these problems. Your proposal to apply a genetic approach to address these questions, while challenging, is appropriate and exciting. As you know, my lab is generating mutants of *Rickettsia prowazekii* and I think many of the techniques and protocols we have developed should be applicable to the *Ehrlichia* as well. As I discussed with you, we are currently targeting specific genes by homologous recombination as well as obtaining random transposon insertions. Both approaches should be very useful for your studies. Also, as Dr. Munderloh and co-workers described for *Anaplasma*, we have successfully obtained expression of GFP_{UV} in *Rickettsia prowazekii* using a *Himar 1* transposase system. Thus, there are a number of genetic tools that are available that can be applied to your ehrlichial studies. Applying genetic techniques to address important questions concerning intracellular parasitism is very exciting and I would be glad to serve as a collaborator on your new proposal.

I will be able to supply you with any additional recombinant plasmids or transposons that you may need. In addition, any protocols that we have developed in our rickettsial work or any appropriate antibiotic resistance genes or transposon constructs that we have found useful will also be available to you. As you can imagine, I am very interested in determining if our approaches for examining rickettsial species will also be useful in other obligate intracellular parasites. I look forward to a productive collaboration. Please let me know if I can supply any additional information.

Sincerely,

Personal Info

Redacted by agreement

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PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

❍ New ● Resubmission ❍ Renewal ❍ Continuation ❍ Revision

Federal Identifier: 1R01 AI070908-01A1

2. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

❑ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ❍ No ❍

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ❍ No ❍

Obtained via FOIA by White Coat Waste Project

Tracking Number:

OMB Number. 0925-0001
Expiration Date: 9/30/2007

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

○Yes ●No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance, where applicable, provide an explanation and attach below.

Explanation:

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Attachments

CertificationExplanation_attDataGroup0

File Name	Mime Type

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Notice of Award

RESEARCH **Issue Date:** 06/09/2009

Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI070908-02 REVISED

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Asst. Vice Provost for Research
PreAward Services
2 Fairchld Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Budget Period: 12/01/2008 – 11/30/2009
Project Period: 12/15/2007 – 11/30/2012

Dear Business Official:

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $36,500 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number R01AI070908 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health."

Award recipients are required to comply with the NIH Public Access Policy. This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator. Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities. Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report. Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award. The NIH website http://grants.nih.gov/grants/policy/coi/index.htm provides additional information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Obtained via FOIA by White Coat Waste Project

Sincerely yours,

Ann W. Devine
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

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NIH NGA R | Version: 368 - 03/24/2009 21:27:26 | Generated on: 06/09/2009 00:27:06

SECTION I – AWARD DATA – 5R01AI070908-02 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$250,000
Federal F&A Costs	$115,000
Approved Budget	$365,000
Federal Share	$365,000
TOTAL FEDERAL AWARD AMOUNT	$365,000

AMOUNT OF THIS ACTION (FEDERAL SHARE) $36,500

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$365,000	$365,000
3	$365,000	$365,000
4	$365,000	$365,000
5	$365,000	$365,000

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908A
Fiscal Year:	2009

IC	*CAN*	*2009*	*2010*	*2011*	*2012*
AI	8472350	$365,000	$365,000	$365,000	$365,000

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C / **OC**: 414E / **Processed**: eRA Commons User Name 06/08/2009

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI070908-02 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI070908-02 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

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In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI070908-02 REVISED

This revised award increases the total costs authorized consistent with the NIH fiscal policy for FY2009 (See NIH Guide Notice NOT-OD-09-066).

Supersedes Notice of Award (NoA) issued 11/12/2008

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Mollie Shea
Email: mshea@niaid.nih.gov **Phone**: 301-402-6576 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 301-402-5083 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI070908-02 REVISED

INSTITUTION: KANSAS STATE UNIVERSITY

Facilities and Administrative Costs	***Year 2***	***Year 3***	***Year 4***	***Year 5***
F&A Cost Rate 1	46%	46%	46%	46%
F&A Cost Base 1	$250,000	$250,000	$250,000	$250,000
F&A Costs 1	$115,000	$115,000	$115,000	$115,000

Obtained via FOIA by White Coat Waste Project

OMB No. 0925-0001

Grant Number 5R01AI70908-2	**Total Project Period** From: 12/15/2007 To: 11/30/2012
EIN: **Review Group:** 1480771751A1 ZRG1 IDM-B (02) M	**Requested Budget Period:** From: 12/01/2008 To: 11/30/2009
Title of Project: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression	**Due Date:** 10/16/2008 **Submitted Date:** 10/16/2008
Program Director/Principal Investigator: ROMAN R. GANTA Diagnostic Med./Pathobiology College of Veterinary Medicine 1800 Denison Ave Manhattan , KS 66506 **Phone Number:** 785-532-4612 **Fax Number:** 785-532-4039 **Email Address:** rganta@vet.ksu.edu	**Applicant Organization:** KANSAS STATE UNIVERSITY KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL MANHATTAN , KS 665061103 **Department:** DIAGNOSTIC MEDICINE/PATHOBIOLOGY **Major Subdivision:** COLLEGE OF VETERINARY MEDICINE
Administrative Official: Carmen J Garcia KANSAS STATE UNIVERSITY 2 Fairchild Hall MANHATTAN , KS 665061103 **Phone Number:** 7855326804 **Fax Number:** 7855325944 **Email Address:** carmen@ksu.edu	**Signing Official:** Paul R Lowe 2 Fairchild Hall Manhattan , KS 665101103 **Phone Number:** 785-532-6804 **Fax Number:** 785-532-5944 **Email Address:** plowe@ksu.edu
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** FWA00000865 **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ Previously Reported ☐ Not Previously Reported
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name: KANSAS STATE UNIVERSITY		
DUNS: 929773554		
Street 1: KANSAS STATE UNIVERSITY	Street 2: 2 FAIRCHILD HALL	
City: MANHATTAN	County: Riley	State: KS
Province:	Country: UNITED STATES	Zip/Postal Code: 665061103
Congressional Districts: 002		

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Program Director/Principal Investigator:	**Grant Number**
ROMAN R. GANTA	5R01AI70908-2
Applicant Organization:	**Period Covered by this Report:**
KANSAS STATE UNIVERSITY	12/15/2007 - 11/30/2008

Title of Project:

Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Citation ID:	**Citation Source:**	**Citation Text:**
18710870	PD/PI Entered	Unpublished
	PD/PI Entered	Unpublished
18770538	PD/PI Entered	Cheng C, Sirigireddy KR, Ganta RR. Isolation and molecular detection of Ehrlichia from vertebrate animals. Curr Protoc Microbiol. 2008 May;Chapter 3:Unit 3A.3.

Obtained via FOIA by White Coat Waste Project

18770537	PD/PI Entered	Cheng C, Ganta RR. Laboratory maintenance of Ehrlichia chaffeensis and Ehrlichia canis and recovery of organisms for molecular biology and proteomics studies. Curr Protoc Microbiol. 2008 May;Chapter 3:Unit 3A.1.
	PD/PI Entered	Unpublished
18245255	PD/PI Entered	Luce-Fedrow A, Von Ohlen T, Boyle D, Ganta RR, Chapes SK. Use of Drosophila S2 cells as a model for studying Ehrlichia chaffeensis infections. Appl Environ Microbiol. 2008 Mar;74(6):1886-91.

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Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

Obtained via FOIA by White Coat Waste Project

Senior/Key Personnel Report					
Program Director/Principal Investigator: ROMAN R. GANTA			**Grant Number** 5R01AI70908-2		
Name:	**Degree(s) Name:**	**SSN:**	**Role on Project:**	**Months Devoted to Project**	
				Cal	**Acad**	**Sum**
ROMAN R. GANTA	PHD, MS, BS	Personal Info	PD/PI	EFFORT		
Redacted by agreement			Other (Specify)-Collaborator			
			Other (Specify)-Collaborator			

Obtained via FOIA by White Coat Waste Project

A. SPECIFIC AIMS: The central *hypothesis* of this study is that *the transcription from the 28 kDa outer membrane protein (p28-Omp) multigene locus in E. chaffeensis is differentially regulated in response to environmental signals and that the host cell-specific gene expression is essential for the pathogen's survival in vertebrate and tick cell environments*. Proposed **specific aims** of our project are: **1)** Establish *in vitro* transcription system for mapping *Ehrlichia* promoters. **2)** Map transcriptional machinery in differentially expressed genes 14 and 19 of the p28-Omp locus. **3)** Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19. **4)** Evaluate knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific expression.

B. STUDIES AND RESULTS: During the first year of the funding period, significant effort was directed in conducting the research on all four aims.

Specific Aim 1: Establish *in vitro* transcription system for mapping *Ehrlichia* promoters.

E. chaffeensis RNA polymerase purification: In our preliminary studies, we reported a method for *in vitro* transcription coupled with translation using *E. coli* RNA polymerase to drive expression from *E. chaffeensis* promoters. Preliminary studies have also been initiated using crude extracts prepared from *E. chaffeensis* cultures. We now extended these studies to obtain pure preparations of *E. chaffeensis* RNA polymerase. Large quantities of the pathogen cultures were grown and used to isolate purified RNA polymerase by following the heparin-agarose purification method adapted with modifications from that described by Chamberlin *et al.* (Methods Enzymol. 101:540-568). *E. chaffeensis* was grown in macrophage cell line, DH82, to infectivity reached nearly 80%. Cultures were harvested, purified by following differential centrifugation methods. Subsequently, *E. chaffeensis* lysates were prepared and RNA polymerase was purified. Heparin agarose purified fractions were resolved and assessed for the presence of purified proteins by PAGE gel analysis followed by silver staining. Peak fractions that contained purified proteins were identified. The protein preparations are currently under investigation to determine if they contain RNA polymerase activity.

In vitro transcription: The *in vitro* transcription assay described in our preliminary data used p28-Omp 14 and 19 promoters to drive expression of lacZ reporter gene activity. Similarly, the analysis was performed to drive the expression of green fluorescence protein. We compiled all these data and the manuscript was submitted for publication to a peer-review journal (Peddireddi et al.). Although we observed predicted transcribed products, we also noted several smaller, non-specific *in vitro* transcribed products. To reduce the non-specific product synthesis, we obtained a G-less cassette, pMT504, described by Dr. Ming Tan's group for *in vitro* transcription assays of *Chlamydia trachomatis* [J. Bact. 178, 6975-6982, (1996)]. We are now modifying the pMT504 for use in testing the promoter activities of p28-Omp 14 and 19 genes.

Specific Aim 2: Map transcriptional machinery in differentially expressed genes of the p28-Omp locus. In our preliminary studies, we mapped the transcription start sites for genes 14 and 19 of the p28-Omp locus by primer extension and RNase protection assays. In our *in vitro* transcription assays, we verified that the transcription is initiated from the same starting point as that observed *in vivo* (Figure 1). These new data validate that the *in vitro* system that we developed simulates that of *in vivo*. We continued studies to identify the minimal core promoter required for the RNA polymerase to drive the transcription for genes 14 and 19. We made several deletion constructs and demonstrated their promoter activity in *E. coli*. To date, we made several deletions from 5' end by employing PCR methods using different 5' end primers with the same 3' end primer used in constructing the genes 14 and 19 promoter constructs. The initial deletions included ~60 base pair deletions from the 5' end. These data were included in our Unpublished We also prepared additional deletion constructs where we deleted the predicted -10 and/or -35 regions for the genes 14 and 19 promoters. In addition, we designed several primer sets useful in preparing point mutations within the -10 and -35 regions. Efforts are now underway to assess the promoter activities of the deletion constructs we prepared.

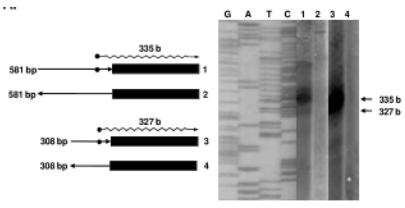

Figure 1. *In vitro* transcription analysis. *In vitro* transcription analysis was performed for the complete upstream sequences of genes 14 and 19 in forward and reverse orientations ligated to a partial lacZ gene segment (301 bp). Genes 14 and 19 sequence segments and the predicted transcripts are shown as cartoons on the left, and the observed transcripts are shown on the right of the panels. Puc18 plasmid DNA was used as the template to generate sequence ladder using an M13 forward primer. Numbers 1 and 2 refer to the constructs for *in vitro* transcription of gene 14 and 3 and 4 refer to *in vitro* transcription templates for gene 19.

Obtained via FOIA by White Coat Waste Project

Specific Aim 3: Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19 of the p28-Omp locus: In support of accomplishing the goals of this aim, we standardized the protocols for mobility shift assay. The assay standardization was performed using ^{32}p-labeled probes of the entire promoter regions of genes 14 and 19. For these assays, *E. chaffeensis* protein preparations were made from the organisms cultured and purified from the DH82 macrophage cell line. We observed shift in the promoter regions when *E. chaffeensis* protein preparations were added. The assays will be modified to use non-radio active probe system and the work is now in progress. The mobility shift assays are the first step towards identifying the regulators associated in modifying gene activities in a host cell-specific manner.

Specific Aim 4: Evaluation of knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific expression.
The goal of this aim is to develop knockout system useful in testing the hypothesis that unique host cell-specific expression sites are essential for the pathogen's survival in vertebrate and tick cell environments. We surveyed the global gene expression of *E. chaffeensis* originating from macrophage and tick cells by performing proteome and microarray analysis. Recently, a manuscript describing the proteome data was accepted for publication and was already published online, ahead of print publication (Infect. Immun.) A manuscript describing the microarray data is also in preparation. The functional genomics analysis aided in identifying several targets for designing knockout mutations. We prepared several recombinant plasmid constructs to create mutations in a gene that is transcriptionally inactive (as judged from our microarry and proteomic studies). Our initial attempts to generate mutations were unsuccessful, but are useful in standardizing many protocols needed for mutational experiments. We made several changes to the construct design and the work is still preliminary at this point.

C. SIGNIFICANCE: Novel observations from this year's study include the demonstration of differences in the global pathogen protein expression profiles and the development of methods for in vitro analysis of gene regulation. The studies will be important to assess the importance of tick transmission of *E. chaffeensis* and how it may contribute to the pathogen's ability to adapt to dual host environments.

D. PLANS: Protocols outlined in the original proposal will be followed during the next year in continue making the progress in all four specific aims of the proposal. Specifically, we will continue our studies to define the *in vitro* transcription assays, fine map promoters of differentially expressed p28-Omp genes, assess host cell contributions to gene functions and knockout system development in *E. chaffeensis*.

E. PUBLICATIONS:
1) Seo GM, Cheng C, Tomich J, Ganta RR. Total, membrane, and immunogenic proteomes of macrophage- and tick cell-derived *Ehrlichia chaffeensis* evaluated by LC-MS/MS and MALDI-TOF methods. ***Infect Immun***. (in press 2008) (2008, Aug 18. Epub ahead of print) PMID: 18710870

Unpublished

3) Cheng C, Sirigireddy KR, Ganta RR. Isolation and molecular detection of *Ehrlichia* from vertebrate animals. ***Curr Protoc Microbiol***. 2008 May;Chapter 3:Unit 3A.3. PMID: 18770538

4) Cheng C, Ganta RR. Laboratory maintenance of *Ehrlichia chaffeensis* and *Ehrlichia canis* and recovery of organisms for molecular biology and proteomics studies. ***Curr Protoc Microbiol***. 2008 May;Chapter 3:Unit 3A.1. PMID: 18770537

Unpublished

6) Luce-Fedrow A, Von Ohlen T, Boyle D, Ganta RR, Chapes SK. Use of Drosophila S2 cells as a model for studying *Ehrlichia chaffeensis* infections. ***Appl Environ Microbiol***. 2008 Mar;74(6):1886-91.

Obtained via FOIA by White Coat Waste Project

Notice of Award

RESEARCH **Issue Date:** 05/03/2010

Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI070908-03 REVISED

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Asst. Vice Provost for Research
PreAward Services
2 Fairchld Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Budget Period: 12/01/2009 – 11/30/2010
Project Period: 12/15/2007 – 11/30/2012

Dear Business Official:

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $32,850 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number R01AI070908 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health."

Award recipients are required to comply with the NIH Public Access Policy. This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator. Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities. Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report. Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award. The NIH website http://grants.nih.gov/grants/policy/coi/index.htm provides additional information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Obtained via FOIA by White Coat Waste Project

Sincerely yours,

Mollie Shea
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Obtained via FOIA by White Coat Waste Project

NIH NGA R | Version: 385 - 11/24/2009 13:33:07 | Generated on: 05/03/2010 19:07:57

SECTION I – AWARD DATA – 5R01AI070908-03 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$247,500
Federal F&A Costs	$113,850
Approved Budget	$361,350
Federal Share	$361,350
TOTAL FEDERAL AWARD AMOUNT	$361,350

AMOUNT OF THIS ACTION (FEDERAL SHARE) $32,850

SUMMARY TOTAL FEDERAL AWARD AMOUNT YEAR (3)	
GRANT NUMBER	**TOTAL FEDERAL AWARD AMOUNT**
5R01AI070908-03	$361,350
3R01AI070908-03S1	$61,917
TOTAL	**$423,267**

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
3	$361,350	$423,267
4	$361,350	$361,350
5	$361,350	$361,350

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908A
Fiscal Year:	2010

IC	*CAN*	*2010*	*2011*	*2012*
AI	8472350	$361,350	$361,350	$361,350

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C / **OC**: 414E / **Processed** eRA Commons User Name 04/30/2010

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI070908-03 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI070908-03 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

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An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI070908-03 REVISED

This revised award increases the total costs authorized consistent with the NIH fiscal policy for FY2010 (See NIH Guide Notice NOT-OD-10-039).

Supersedes Notice of Award (NoA) issued 11/12/09.
****
NIH is currently funded through a Continuing Resolution at the FY2009 level (See NIH Guide Notice NOT-OD-10-012: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-012.html. Therefore, this non-competing award has been made at a level below that indicated in the Notice of Grant Award. If the final appropriation permits, adjustments may be made up to the FY2010 funding plan level.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Mollie Shea
Email: mshea@niaid.nih.gov **Phone**: 301-402-6576 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 301-402-5083 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI070908-03 REVISED

INSTITUTION: KANSAS STATE UNIVERSITY

Facilities and Administrative Costs	***Year 3***	***Year 4***	***Year 5***
F&A Cost Rate 1	46%	46%	46%
F&A Cost Base 1	$247,500	$247,500	$247,500
F&A Costs 1	$113,850	$113,850	$113,850

Obtained via FOIA by White Coat Waste Project

OMB No. 0925-0001

Grant Number 5R01AI70908-3	**Total Project Period** From: 12/15/2007 To: 11/30/2012
EIN: **Review Group:** 1480771751A1 ZRG1 IDM-B (02) M	**Requested Budget Period:** From: 12/01/2009 To: 11/30/2010
Title of Project: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression	**Due Date:** 10/16/2009 **Submitted Date:** 10/16/2009
Program Director/Principal Investigator: ROMAN R. GANTA Diagnostic Med./Pathobiology College of Veterinary Medicine 1800 Denison Ave Manhattan , KS 66506 **Phone Number:** 785-532-4612 **Fax Number:** 785-532-4039 **Email Address:** rganta@vet.k-state.edu	**Applicant Organization:** KANSAS STATE UNIVERSITY KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL MANHATTAN , KS 665061103 **Department:** DIAGNOSTIC MEDICINE/PATHOBIOLOGY **Major Subdivision:** COLLEGE OF VETERINARY MEDICINE
Administrative Official: Carmen J Garcia KANSAS STATE UNIVERSITY 2 Fairchild Hall MANHATTAN , KS 665061103 **Phone Number:** 7855326804 **Fax Number:** 7855325944 **Email Address:** carmen@ksu.edu	**Signing Official:** Anita S Fahrny 2 Fairchild Hall Kansas State University Manhattan , KS 665061103 **Phone Number:** 785-532-6804 **Fax Number:** **Email Address:** afahrny@ksu.edu
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** FWA00000865 **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ Previously Reported ☒ Not Previously Reported
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name: KANSAS STATE UNIVERSITY		
DUNS: 929773554		
Street 1: KANSAS STATE UNIVERSITY	Street 2: 2 FAIRCHILD HALL	
City: MANHATTAN	County: Riley	State: KS
Province:	Country: UNITED STATES	Zip/Postal Code: 665061103
Congressional Districts: 002		

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Program Director/Principal Investigator:	**Grant Number**
ROMAN R. GANTA	5R01AI70908-3
Applicant Organization:	**Period Covered by this Report:**
KANSAS STATE UNIVERSITY	12/01/2008 - 11/30/2009

Title of Project:

Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

SNAP Questions:

Has there been a change in the other support of All Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other All Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID:	Citation ID:	Citation Source:	Citation Text:
	18710870	PD/PI Entered	Seo GM, Cheng C, Tomich J, Ganta RR. Total, membrane, and immunogenic proteomes of macrophage- and tick cell-derived Ehrlichia chaffeensis evaluated by LC-MS/MS and MALDI-TOF methods. Infect Immun. (in press 2008) (2008, Aug 18. Epub ahead of print)
	18770538	PD/PI Entered	Cheng C, Sirigireddy KR, Ganta RR. Isolation and molecular detection of Ehrlichia from vertebrate

Obtained via FOIA by White Coat Waste Project

			animals. Curr Protoc Microbiol. 2008 May;Chapter 3:Unit 3A.3.
	18770537	PD/PI Entered	Cheng C, Ganta RR. Laboratory maintenance of Ehrlichia chaffeensis and Ehrlichia canis and recovery of organisms for molecular biology and proteomics studies. Curr Protoc Microbiol. 2008 May;Chapter 3:Unit 3A.1.
	18245255	PD/PI Entered	Luce-Fedrow A, Von Ohlen T, Boyle D, Ganta RR, Chapes SK. Use of Drosophila S2 cells as a model for studying Ehrlichia chaffeensis infections. Appl Environ Microbiol. 2008 Mar;74(6):1886-91.
Yes	82685	NIHMS	Authors: Chapes SK, Ganta RR Title: Defining the immune response to Ehrlichia species using murine models. Date: 12/20/2008 Type: reference

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Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

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All Personnel Report

Program Director/Principal Investigator:	Grant Number
ROMAN R. GANTA	5R01AI70908-3

Name:	Commons ID:	Degree(s) Name:	SSN:			
ROMAN R. GANTA		PHD, MS, BS	Personal Info	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
PD/PI			Personal Info	EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Redacted by agreement		BS	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
Technician				EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Redacted by agreement		BS	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
Research Associate				EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Redacted by agreement		BS	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
Graduate Research Assistant				EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Redacted by agreement		BS	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
Graduate Research Assistant				EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:

Obtained via FOIA by White Coat Waste Project

Redacted by agreement		PHD	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
Research Assistant/Coordinator				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement			XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
Other (Specify)-Collaborator				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement			XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
Undergraduate Research Assistant				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement		BS	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
Graduate Research Assistant				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement			XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/DD)	**Cal**	**Acad**	**Sum**
Research Assistant/Coordinator				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement			XXX-XX-	Months Devoted to Project		

Obtained via FOIA by White Coat Waste Project

Role on Project:	Supplement Support:	DoB: (MM/DD)	Cal	Acad	Sum
Other (Specify)-Collaborator			EFFORT		

Obtained via FOIA by White Coat Waste Project

A. SPECIFIC AIMS: The central *hypothesis* of this study is that *the transcription from the 28 kDa outer membrane protein (p28-Omp) multigene locus in E. chaffeensis is differentially regulated in response to environmental signals and that the host cell-specific gene expression is essential for the pathogen's survival in vertebrate and tick cell environments*. Proposed **specific aims** are: **1)** Establish *in vitro* transcription system for mapping *Ehrlichia* promoters. **2)** Map transcriptional machinery in differentially expressed genes 14 and 19 of the p28-Omp locus. **3)** Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19. **4)** Evaluate knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific expression.

B. STUDIES AND RESULTS: During this year of the funding period, significant effort was directed in conducting the research on all four aims.

Specific Aim 1: Establish *in vitro* transcription system for mapping *Ehrlichia* promoters.
E. chaffeensis RNA polymerase purification: Large quantities of the pathogen cultures were grown and used to isolate *E. chaffeensis* RNA polymerase by following the heparin-agarose purification method. We needed to introduce considerable modifications to the Chamberlin *et al.* (Methods Enzymol. 101:540-568) method to recover active enzyme. *E. chaffeensis* was grown in macrophage cell line, DH82, to infectivity reached nearly 80%. Cultures were harvested and purified free of the host cells by following lysis and differential centrifugation methods. Subsequently, *E. chaffeensis* lysates were prepared and RNA polymerase was purified by the heparin agarose methods. Peak fractions that contained purified proteins were utilized in performing in vitro transcription assays. The enzyme was characterized to identify optimal conditions of the divalent and monovalent cation specificity. The bacterial origin of the holoenzyme was confirmed by employing immunoblotting analysis with *E. coli* Sigma 70 antibody (which is known to cross react with several Gram negative bacterial RNA polymerases). Furthermore, the enzyme specificity was confirmed by its rifampin and *E. coli* sigma 70 antibody-mediated inhibition of enzyme activity. The detailed enzyme characterization is also described at a recent American Society for Rickettsiology conference.

In vitro transcription: The *in vitro* transcription assay has been further improved this year. Several G-less cassettes were prepared with *E. chaffeensis* promoter sequences. The G-less cassettes containing the E. chaffeensis differentially expressed gene promoters of p28-Omp 14 and 19 genes were prepared and used in performing in vitro transcription assays. The constructs worked well with *E. coli* RNA polymerase. The constructs were also utilized in the modified assays with *E. chaffeensis* RNA polymerase (purified as described above).

Recombinant Sigma 70 and Sigma 32 subunits of *E. chaffeensis* RNA polymerase: As a backup plan to native *E. chaffeensis* RNA polymerase purification, we also constructed expression plasmid constructs containing Sigma 70 and Sigma 32 subunits of *E. chaffeensis* RNA polymerase. We successfully cloned and expressed these subunits in *E. coli* using pET32 expression system (Novagen). The recombinant Sigma 70 protein was combined with *E. coli* core enzyme and tested and found to be active in driving expression from *E. chaffeensis* gene promoters. (*E. coli* core enzyme alone is negative for RNA polymerase activity. Specifically, it alone did not drive the expression from *E. chaffeensis* gene promoters.) Further, recombinant *E. chaffeensis* Sigma 70-driven enzyme properties were assessed and found to be very similar to those of native, purified enzyme. The Sigma 32 work is currently in progress. Similarly, efforts are underway to identify specificities of several *E. chaffeensis* promoters for their specificities to Sigma 32- and Sigma 70-specific RNA polymerases.

Specific Aim 2: Map transcriptional machinery in differentially expressed genes of the p28-Omp locus. We already made considerable progress in support of this specific aim last year (manuscript published). Efforts are now underway to assess the promoter activities of the deletion constructs using in vitro assays we developed and using the native and recombinant *E. chaffeensis* RNA polymerase. In support of these experiments, we prepared several deletion constructs to drive expression from G-less cassettes. Work is still ongoing and we anticipate significant progress in the upcoming year.

Specific Aim 3: Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19 of the p28-Omp locus: In support of accomplishing the goals of this aim, we already standardized several important protocols for mobility shift experiments

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(described in last year's progress report). This year, we expressed several putative regulators as recombinant proteins in *E. coli*. The recombinant proteins were purified and efforts are now underway to assess their interactions with the promoter regions of differentially expressed outer membrane proteins genes, p28Omp 14 and 19.

Specific Aim 4: Evaluation of knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific expression. The goal of this aim is to develop knockout system useful in testing the hypothesis that unique host cell-specific expression sites are essential for the pathogen's survival in vertebrate and tick cell environments. The functional genomics analysis (reported in our recent publications; listed in the last year's progress report) aided in identifying several targets for designing knockout mutations. We prepared several recombinant plasmid constructs to create mutations. We already made numerous attempts to create mutations by homologous recombination strategy. Our efforts included the development of several constructs containing variable lengths of *E. chaffeensis* genome target DNA segments and promoters to drive expression of inserted antibiotics cassettes. Despite numerous attempts, to date we were not successful in generating mutations in *E. chaffeensis*. We have been actively pursuing these efforts and making modifications to our protocols to the construct design, transformation and to monitor mutations. The work is still ongoing and we anticipate making progress in the upcoming year.

C. SIGNIFICANCE: Novel observations from this year's study include the establishment of methods to purify native and recombinant *E. chaffeensis* RNA polymerase and the refinement of methods for in vitro analysis of gene regulation. The availability of in vitro methods to assess the importance of differential expression and how *E. chaffeensis* regulates its gene expression are important for the understanding the pathogenicity, its evasion of host response and adaptation to dual host environments. We are the first to purify and characterize an *Ehrlichia* RNA polymerase. We expect that these studies will lead the way to parallel research in other related tick-borne rickettsial pathogens.

D. PLANS: We will continue our work on all four specific aims of the proposal. We will continue our studies to define the *in vitro* transcription assays, fine map promoters of differentially expressed p28-Omp genes using *E. chaffeensis* RNA polymerase, assess host cell contributions to gene functions and to continue studies to develop knockout system for *E. chaffeensis*.

E. PUBLICATIONS:

Promoter analysis of macrophage- and tick cell-specific differentially expressed *Ehrlichia chaffeensis* p28-Omp genes. Peddireddi L, Cheng C, Ganta RR.
BMC Microbiol. 2009 May 19;9:99.
PMID: 19454021

Molecular characterization of *Ehrlichia* interactions with tick cells and macrophages.
Ganta RR, Peddireddi L, Seo GM, Dedonder SE, Cheng C, Chapes SK.
Front Biosci. 2009 Jan 1;14:3259-73.
PMID: 19273271

Unpublished

Unpublished

Obtained via FOIA by White Coat Waste Project

F. ABSTRACTS:

Promoter Analysis of Macrophage- and Tick Cell-specific Differentially Expressed *Ehrlichia chaffeensis* p28-Omp Genes. Lalitha Peddireddi, Chuanmin Cheng and Roman R. Ganta
The 23rd Meeting of the American Society for Rickettsiology. August 15-18, 2009, Hilton Head Island, SC, USA (Abstract #8)

In vitro Transcriptional Analysis of *Ehrlichia chaffeensis* Differentially Expressed Genes. Bonto Faburay, Huitao Liu, and Roman Ganta
The 23rd Meeting of the American Society for Rickettsiology. August 15-18, 2009, Hilton Head Island, SC, USA (Abstract #9)

Evaluation of Methods for Establishing Mutations in *E. chaffeensis* by Homologous Recombination. Chuanmin Cheng and Roman R. Ganta
The 23rd Meeting of the American Society for Rickettsiology. August 15-18, 2009, Hilton Head Island, SC, USA (Abstract #18)

Ultrastructural Variations of *Ehrlichia chaffeensis* in Macrophages and Tick Cells. Sarah E. Dedonder, Lloyd Willard, Chuanmin Cheng, and Roman R. Ganta
The 23rd Meeting of the American Society for Rickettsiology. August 15-18, 2009, Hilton Head Island, SC, USA (Abstract #109)

Obtained via FOIA by White Coat Waste Project

Notice of Award

RESEARCH **Issue Date:** 04/19/2010

Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 3R01AI070908-03S1

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Asst. Vice Provost for Research
PreAward Services
2 Fairchld Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Budget Period: 05/01/2010 – 11/30/2010
Project Period: 12/15/2007 – 11/30/2012

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $61,917 (see “Award Calculation” in Section I and “Terms and Conditions” in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the “Terms and Conditions” is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as “The project described was supported by Award Number R01AI070908 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health.”

Award recipients are required to comply with the NIH Public Access Policy. This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator. Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities. Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report. Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award. The NIH website http://grants.nih.gov/grants/policy/coi/index.htm provides additional information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Obtained via FOIA by White Coat Waste Project

Sincerely yours,

Mollie Shea
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Obtained via FOIA by White Coat Waste Project

NIH NGA R | Version: 385 - 11/24/2009 13:33:07 | Generated on: 04/19/2010 00:04:22

SECTION I – AWARD DATA – 3R01AI070908-03S1

Award Calculation (U.S. Dollars)

Federal Direct Costs	$61,917
Approved Budget	$61,917
Federal Share	$61,917
TOTAL FEDERAL AWARD AMOUNT	$61,917
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$61,917

SUMMARY TOTAL FEDERAL AWARD AMOUNT YEAR (3)	
GRANT NUMBER	**TOTAL FEDERAL AWARD AMOUNT**
3R01AI070908-03S1	$61,917
5R01AI070908-03	$328,500
TOTAL	**$390,417**

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
3	$61,917	$390,417
4	$0	$365,000
5	$0	$365,000

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908A
Fiscal Year:	2010

IC	*CAN*	*2010*
AI	8472305	$61,917

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C / **OC**: 414C / **Processed** eRA Commons User Name 04/14/2010

SECTION II – PAYMENT/HOTLINE INFORMATION – 3R01AI070908-03S1

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 3R01AI070908-03S1

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

Obtained via FOIA by White Coat Waste Project

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 3R01AI070908-03S1

This award provides supplemental funds of $61,917.00. Total Costs ($61,917 Direct Costs and $0 F&A Costs) to purchase equipment to support the critical goal of Ehr1ichia RNA polymerase purification and to support all aspects of the project. These funds provide support for the period 05/01/2010 ? 11/30/2010 These funds are restricted for stated purpose, in request dated 04/05/10 from Paul R. Lowe, Assistant Vice President for Research, PreAward Services, Kansas State University and may not be rebudgeted or used for any other purpose, without NIAID awarding unit approval.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Mollie Shea
Email: mshea@niaid.nih.gov **Phone**: 301-402-6576 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 301-402-5083 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 3R01AI070908-03S1

INSTITUTION: KANSAS STATE UNIVERSITY

Obtained via FOIA by White Coat Waste Project

Grant Number: 5R01AI070908-04 REVISED

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Anita S. Fahrny
Asst. Vice Provost for Research
PreAward Services
2 Fairchld Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Budget Period: 12/01/2010 – 11/30/2011
Project Period: 12/15/2007 – 11/30/2012

Dear Business Official:

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $32,522 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number R01AI070908 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health."

Award recipients are required to comply with the NIH Public Access Policy. This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator. Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities. Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report. Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award. The NIH website http://grants.nih.gov/grants/policy/coi/index.htm provides additional information.

Obtained via FOIA by White Coat Waste Project

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Leslie D. Boggs
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Obtained via FOIA by White Coat Waste Project

SECTION I – AWARD DATA – 5R01AI070908-04 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$245,025
Federal F&A Costs	$112,712
Approved Budget	$357,737
Federal Share	$357,737
TOTAL FEDERAL AWARD AMOUNT	$357,737

AMOUNT OF THIS ACTION (FEDERAL SHARE) $32,522

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
4	$357,737	$357,737
5	$357,737	$357,737

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908A
Fiscal Year:	2011

IC	*CAN*	*2011*	*2012*
AI	8472350	$357,737	$357,737

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C / **OC**: 414E / **Processed**: eRA Commons User Name 06/01/2011

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI070908-04 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI070908-04 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

Obtained via FOIA by White Coat Waste Project

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award may be subject to the Transparency Act subaward and executive compensation reporting requirements of 2 CFR Part 170. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI070908-04 REVISED

This revised award increases the total costs authorized consistent with NIAID implementation of the NIH fiscal policy for FY2011 (See NIH Guide Notice NOT-OD-11-068). Future year recommended levels also have been adjusted consistent with the referenced Notice.

Supersedes Notice of Award (NoA) issued 11/05/2010.
****

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Devon R. Bumbray-quarles
Email: dbumbray@niaid.nih.gov **Phone**: 301-402-6213 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 301-402-5083 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI070908-04 REVISED

INSTITUTION: KANSAS STATE UNIVERSITY

Budget	*Year 4*	*Year 5*
TOTAL FEDERAL DC	$245,025	$245,025
TOTAL FEDERAL F&A	$112,712	$112,712
TOTAL COST	$357,737	$357,737

Facilities and Administrative Costs	*Year 4*	*Year 5*
F&A Cost Rate 1	46%	46%
F&A Cost Base 1	$245,025	$245,025
F&A Costs 1	$112,712	$112,712

Obtained via FOIA by White Coat Waste Project

OMB No. 0925-0001

Grant Number 5R01AI70908-4	**Total Project Period** From: 12/15/2007 To: 11/30/2012
EIN: **Review Group:** 1480771751A1 ZRG1 IDM-B (02) M	**Requested Budget Period:** From: 12/01/2010 To: 11/30/2011
Title of Project: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression	**Due Date:** 10/16/2010 **Submitted Date:** 10/18/2010

Program Director/Principal Investigator: ROMAN R. GANTA Diagnostic Med./Pathobiology College of Veterinary Medicine 1800 Denison Ave Manhattan , KS 66506 **Phone Number:** 785-532-4612 **Fax Number:** 785-532-4039 **Email Address:** rganta@vet.k-state.edu	**Applicant Organization:** KANSAS STATE UNIVERSITY KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL MANHATTAN , KS 665061103 **Department:** DIAGNOSTIC MEDICINE PATHOBIOLOGY **Major Subdivision:** COLLEGE OF VETERINARY MEDICINE
Administrative Official: Carmen J Garcia KANSAS STATE UNIVERSITY 2 Fairchild Hall MANHATTAN , KS 665061103 **Phone Number:** 7855326804 **Fax Number:** 7855325944 **Email Address:** carmen@ksu.edu	**Signing Official:** Anita S Fahrny 2 Fairchild Hall Kansas State University Manhattan , KS 665061103 **Phone Number:** 785-532-6804 **Fax Number:** **Email Address:** afahrny@ksu.edu

Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** FWA00000865 **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ Previously Reported ☒ Not Previously Reported
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location

Organizational Name: Kansas State University		
DUNS: 929773554		
Street 1: 2 Fairchild Hall	Street 2:	
City: Manhattan	County:	State: KS
Province:	Country: UNITED STATES	Zip/Postal Code: 665061103
Congressional Districts: KS-002		

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Program Director/Principal Investigator:	Grant Number
ROMAN R. GANTA	5R01AI70908-4
Applicant Organization:	**Period Covered by this Report:**
KANSAS STATE UNIVERSITY	12/01/2009 - 11/30/2010

Title of Project:

Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ No ☐ Yes

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ No ☐ Yes

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ No ☐ Yes

Justification:

Changes in Select Agent Research? ☒ No ☐ Yes

Changes in Multiple PD/PI Leadership plan? ☒ No ☐ Yes

Change in human embryonic stem cell (hESC) line(s) used? ☒ No ☐ Yes

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ No ☐ Yes

Has the Involvement of Animal Subjects changed since previous submission? ☒ No ☐ Yes

Publications:

Valid NIHMSID: **Citation ID:** **Citation Source:** **Citation Text:**

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Cover Letter: File is not uploaded
Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

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All Personnel Report

Program Director/Principal Investigator:	Grant Number
ROMAN R. GANTA	5R01AI70908-4

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
ROMAN R. GANTA	eRA Commons User Name	PHD, MS, BS	Personal Info			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			Personal Info	EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Redacted by agreement		BS	XXX-XX-			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Technician				EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Redacted by agreement		BS	XXX-XX-			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Research Associate				EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Redacted by agreement		BS	XXX-XX-			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Graduate Research Assistant				EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Redacted by agreement		BS	XXX-XX-			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Graduate Research Assistant				EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:

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Redacted by agreement		PHD	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Research Assistant/Coordinator				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement			XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Undergraduate Research Assistant				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement			XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Other (Specify)-Collaborator				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement			XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Undergraduate Research Assistant				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement		BS	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Graduate Research Assistant				EFFORT		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement			XXX-XX-	Months Devoted to Project		

Obtained via FOIA by White Coat Waste Project

Role on Project:	Supplement Support:		DoB: (MM/YY)	Cal	Acad	Sum
Research Assistant/Coordinator				EFFORT		

Name:	Commons ID:	Degree(s) Name:	SSN:		
Redacted by agreement			XXX-XX-	Months Devoted to Project	

Role on Project:	Supplement Support:	DoB: (MM/YY)	Cal	Acad	Sum
Other (Specify)-Collaborator			EFFORT		

Obtained via FOIA by White Coat Waste Project

A. SPECIFIC AIMS: The central hypothesis of this study is that the transcription from the 28 kDa outer membrane protein (p28-Omp) multigene locus in *E. chaffeensis* is differentially regulated in response to environmental signals and that the host cell-specific gene expression is essential for the pathogen's survival in vertebrate and tick cell environments. Proposed **specific aims** are: **1)** Establish *in vitro* transcription system for mapping *Ehrlichia* promoters. **2)** Map transcriptional machinery in differentially expressed genes 14 and 19 of the p28-Omp locus. **3)** Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19. **4)** Evaluate knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific expression.

B. STUDIES AND RESULTS:

Specific Aim 1: Establish *in vitro* transcription system for mapping *Ehrlichia* promoters.

E. chaffeensis RNA polymerase purification: Considerable progress has been made in accomplishing the objectives of this aim. In last year's report, we outlined the establishment of the methods to purify native *E. chaffeensis* RNA polymerase by following heparin-agarose purification method. We adapted the bacterial RNA polymerase purification method with several modifications to the protocol reported by Chamberlin *et al.* (Methods Enzymol. 101:540-568). *E. chaffeensis* RNA polymerase was purified from the organisms grown in the canine macrophage cell line, DH82. Cultures were harvested and purified free of the host cells by following lysis and differential centrifugation methods. Subsequently, *E. chaffeensis* lysates were prepared and RNA polymerase was purified by subjecting to heparin agarose column chromatography. The activity in purified enzyme was confirmed by performing *in vitro* transcription assays for promoter regions of p28-Omp 14 and p28-Omp 19 genes. As described in the previous progress report, optimal conditions for assaying the enzyme activity were also established. To confirm that the enzyme is indeed purified from *E. chaffeensis*, but not represented the RNA polymerase from canine macrophages, two experiments were performed. In the first experiment, we used *E. coli* sigma 70 antibody which is known to cross react with other Gram negative bacterial RNA polymerases to identify the presence of *E. chaffeensis* sigma 70 by Western blot analysis. Secondly, we demonstrated that the antibody inhibited the enzyme activity completely. A manuscript describing the characterization of *E. chaffeensis* RNA polymerase has been submitted recently to a peer-reviewed scientific journal, Unpublished (manuscript is in review).

In vitro transcription: Considerable progress has already been made in establishing the *in vitro* transcription assays and was reported last year. During this year, we made several additional deletion analysis constructs of two differentially expressed gene promoter constructs and tested for their promoter activity under *in vitro*. Several additional genes were also assessed by primer extension analysis and semi-quantitative RT-PCR to identify transcriptionally active genes expressed by sigma 70 and an alternate sigma factor (sigma 32)-dependent RNA polymerases. Furthermore, *in vitro* constructs in a G-less cassette containing plasmid were prepared and used to map the transcriptional activity under *in vitro* conditions. The studies utilized the purified native *E. chaffeensis* RNA polymerase which contained sigma 70 subunit. In addition, we used *E. coli* core enzyme reconstituted with the recombinant *E. chaffeensis* sigma 70 and sigma 32 subunits (described in the next paragraph). This work is in progress and will continue through the next year funding period. Research results from the p28-Omp constructs with several promoter deletion segments were presented at the recent American Society for Rickettsiology meeting held at Stevenson, WA from July 31-August 3, 2010 (abstract #105 by H. Liu et al.). These data will be compiled in a Unpublished Unpublished

Recombinant Sigma 70 and Sigma 32 subunits of *E. chaffeensis* RNA polymerase: In addition to using the native purified *E. chaffeensis* RNA polymerase, we constructed expression plasmids containing Sigma 70 and Sigma 32 subunits of *E. chaffeensis* RNA polymerase. Expression of these proteins, purification and their use for *in vitro* transcription analyses have already been described in the last year's progress report. The data demonstrating that the *E. coli* core enzyme reconstituted with recombinant *E. chaffeensis* Sigma 70 subunit is very similar to the purified native pathogen enzyme. (These data were included in the manuscript submitted t Unpublished described above.) During this funding year, we performed experiments to identify several genes that are transcribed by the RNA polymerase containing Sigma 32 subunit. For these studies, we utilized the recombinant Sigma 32 reconstituted with core enzyme

Obtained via FOIA by White Coat Waste Project

of *E. coli* RNA polymerase. These studies suggested that the *E. chaffeensis* Sigma 32 also recognizes the p28-Omp 14 and 19 gene promoters. In addition, we evaluated several stress-responsive gene promoters; some of them were recognized by the RNA polymerase containing *E. chaffeensis* Sigma 32 subunit. This work is ongoing and we expect to continue defining the contributions of Sigma 32 and 70 subunits in the differential gene expression of *E. chaffeensis*.

Specific Aim 2: Map transcriptional machinery in differentially expressed genes of the p28-Omp locus. We already made considerable progress in support of this specific aim and was reported in the progress report of 2008. This year, we assessed the promoter activities of several deletion constructs using *in vitro* assays using the native and recombinant *E. chaffeensis* RNA polymerase. The differences in the enzyme activities were compared and the data were discussed at the 2010 ASR meeting as indicated above (abstract # 105). This year, we initiated another approach to map the transcriptional machinery of *E. chaffeensis*; we initiated experiments to engineer *E. chaffeensis* Sigma 32 and 70 subunits in *E. coli* mutants lacking its native Sigma 32 and 70 subunits, respectively. The *E. coli* strains will then be transformed with plasmids containing *E. chaffeensis* promoters with a reporter protein coding sequences inserted downstream to the promoter sequences. The goal here is that *E. chaffeensis* homologs complement in *E. coli* mutants lacking its native Sigma subunits. In particular, the expression of *E. chaffeensis* Sigma subunit proteins in *E. coli* are expected to act on *E. chaffeensis* gene promoters and induce the expression of the reporter protein. This will be a novel approach for the rickettsial field. To date, we prepared constructs for experiments to assess *E. chaffeensis* Sigma 32 driven genes. During the next year of funding, we anticipate making considerable progress in this novel approach which helps to characterize *Ehrlichia* promoters in *E. coli*.

Specific Aim 3: Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19 of the p28-Omp locus: In support of accomplishing the goals of this aim, we already standardized several important protocols for mobility shift experiments (described in the previous progress reports). The influence of *E. chaffeensis* protein extracts has been tested and the data were incorporated in the manuscript submitted Unpublished. Several putative regulators were expressed as recombinant proteins in *E. coli*. The recombinant proteins were purified and they will be used to assess their impact on the p28Omp 14 and 19 promoters as well as on other gene promoters as part of next year funded research. More detailed refinement of this study will be undertaken during the next two years of research.

Specific Aim 4: Evaluation of knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific expression. The goal of this aim is to develop knockout systems useful in testing the hypothesis that unique host cell-specific expression sites are essential for the pathogen's survival in vertebrate and tick cell environments. The functional genomics analysis (reported in earlier progress reports) aided in identifying several targets for designing knockout mutations. Last year, we reported that we prepared several recombinant plasmid constructs to create mutations and made numerous attempts to create mutations by homologous recombination strategy. The constructs included four genomic regions targeting to Ech_0126 gene by homologous recombination approach; 6 constructs targeting the coding sequences of three genes (Ech_0126, Ech_1136 and Ech_1143) by utilizing the modified group II intron strategy (Targetron based mutagenesis strategy) and 6 more Targetron constructs for creating mutations in the 5' non-coding regions of genes Ech_0040, Ech_0112 and Ech_0252. The constructs were then evaluated for introducing mutations in *E. chaffeensis* cultured in canine macrophage cell line, DH82. Transformants with mutations were identified in two of the three gene coding sequences of Ech_0126 by both homologous and Targetron-based methods and in Ech_1143 (the p28-Omp 19 gene) by the Targetron method and one in the 5' non-coding regions of Ech_0112 gene (also by Targetron method). The mutations were not detected for other genes, including the Ech_1136 (the p28-Omp 14 gene). The mutations were confirmed by PCR with primers targeting to insertion regions and the genomic regions upstream and downstream to the insertion sites. Mutants were also verified by Southern blot and/or sequencing analysis for a subset of transformants. The mutants were detectable in culture for up to 8 days, however, the establishment of stable transformants remained a challenge. This work was presented at the recent conference of the American Society for Rickettsiology (July31-August 3, 2010; abstract #45). This work is still in progress. In our efforts to improve the transforming efficiency, to monitor the transformants and to

Obtained via FOIA by White Coat Waste Project

identify stable transformants, we have been making additional constructs from different genomic regions and also by including a green fluorescent protein gene to serve as additional marker. Efforts are also underway to create mutations by employing transposon-based mutational methods. These studies will continue as part of the coming year's planned research.

C. SIGNIFICANCE: Progress from this year's study include the refinement of methods to purify native RNA polymerase and the use of recombinant *E. chaffeensis* RNA polymerase Sigma subunits for use in the reconstitution of the functional enzyme. The enzymes are used for *in vitro* mapping of promoters of the pathogen genes and to assess the contributions of host cell environments for altering the gene expression by *E. chaffeensis*. The studies also included the progress towards establishing mutational methods for *E. chaffeensis*. These studies are important for the understanding the pathogenicity caused by this tick-borne rickettsial organism, its evasion of host response and adaptation to dual host environments. Many new methods are developed or in the process of being developed through this project. We expect that these studies will lead the way for parallel research in other related tick-borne rickettsial pathogens of importance to human and animal health.

D. PLANS: We will continue our work on all four specific aims of the proposal. We will continue our studies to further refine the *in vitro* transcription assays and use them to study the regulation of gene expression of *E. chaffeensis* and how host cell environments influence the pathogen protein expression via mediating the regulation of gene expression. Studies also will continue in developing a knockout system to develop stable mutants and to assess the function of differentially expressed proteins of *E. chaffeensis*.

E. PUBLICATIONS (published or submitted):

Unpublished

Comparison of iatrogenic transmission of *Anaplasma marginale* in Holstein steers via needle and needle-free injection techniques. Reinbold JB, Coetzee JF, Hollis LC, Nickell JS, Riegel CM, Christopher JA, Ganta RR. Am J Vet Res. 2010 Oct;71(10):1178-88. PMID: 20919904

Detection of *Anaplasma marginale* and *A. phagocytophilum* in bovine peripheral blood samples by duplex real-time reverse transcriptase PCR assay. Reinbold JB, Coetzee JF, Sirigireddy KR, Ganta RR. J Clin Microbiol. 2010 Jul;48(7):2424-32. Epub 2010 May 12. PMID: 20463162

The efficacy of three chlortetracycline regimens in the treatment of persistent *Anaplasma marginale* infection. Reinbold JB, Coetzee JF, Hollis LC, Nickell JS, Riegel C, Olson KC, Ganta RR. Vet Microbiol. 2010 Sep 28;145(1-2):69-75. Epub 2010 Mar 3. PMID: 20346598

F. ABSTRACTS:

Preparation of a Recombinant Sigma 70 Subunit of the Ehrlichia chaffeensis RNA Polymerase and Its Application in Mapping the Promoter Regions of the p28-outer Membrane Protein Genes, 14 and 19 Huitao Liu*, Bonto Faburay and Roman R. Ganta. The 24th meeting of the American Society for Rickettsiology. July 31-August 3, 2010 (Abstract #105)

Evaluation of Methods for Establishing Mutations in Ehrlichia chaffeensis by Homologous Recombination and Targetron Based Mutagenesis. Chuanmin Cheng, Shanzhong Gong and Roman R. Ganta. The 24th meeting of the American Society for Rickettsiology. July 31-August 3, 2010 (Abstract #45)

Structural analysis of tick- and macrophage-specific differentially expressed Ehrlichia chaffeensis outer membrane proteins, p28 Omp-14 and p28 Omp-19 Gangadaar Thotakura*, Daisuke Takahashi, Om Prakash and Roman R. Ganta. The 24th meeting of the American Society for Rickettsiology. July 31-August 3, 2010 (Abstract #81)

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Grant Number: 5R01AI070908-05 REVISED

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Anita S. Fahrny
Asst. Vice Provost for Research
PreAward Services
2 Fairchld Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Budget Period: 12/01/2011 – 08/11/2013
Project Period: 12/15/2007 – 08/11/2013

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI070908. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Gregory P. Smith
Grants Management Officer

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

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NIH NGA R | Version: 431 - 02/06/2013 13:43:00 | Generated on: 08/22/2013 00:07:13

SECTION I – AWARD DATA – 5R01AI070908-05 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$245,025
Federal F&A Costs	$112,712
Approved Budget	$357,737
Federal Share	$357,737
TOTAL FEDERAL AWARD AMOUNT	$357,737

AMOUNT OF THIS ACTION (FEDERAL SHARE) $0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
5	$357,737	$357,737

Fiscal Information:

CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908A
Fiscal Year:	2012

IC	*CAN*	*2012*
AI	8472350	$357,737

NIH Administrative Data:
PCC: M46C / **OC**: 414E / **Released**: eRA Commons User Name 08/21/2013
Award Processed: 08/22/2013 12:07:13 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI070908-05 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI070908-05 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See

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http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/#gps .

A final Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 90 days of the expiration date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) cash transaction data.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 90 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

Unless an application for competitive renewal is submitted, a final progress report must also be submitted within 90 days of the expiration date. Instructions for preparing a Final Progress Report are at: http://grants.nih.gov/grants/funding/finalprogressreport.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report. Institute/Centers may accept the progress report contained in competitive renewal (type 2) in lieu of a separate final progress report. Contact the awarding IC for IC-specific policy regarding acceptance of a progress report contained in a competitive renewal application in lieu of a separate final progress report.

NIH **strongly encourages** electronic submission of the final progress report and the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final progress report and final invention statement may be e-mailed as PDF attachments to the NIH Central Closeout Center at: DeasCentralized@od.nih.gov.

Hard copy: Paper submissions of the final progress report and the final invention statement may be faxed to the NIH Division of Central Grants Processing at 301-480-2304, or mailed to:

NIH Division of Central Grants Processing, OER
6705 Rockledge Drive
Suite 5016, Room 5109
MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final Progress Report is not required. However, a final FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

Treatment of Program Income:
Additional Costs

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SECTION IV – AI Special Terms and Conditions – 5R01AI070908-05 REVISED

REVISED AWARD: This term and condition of award is revised to correct the budget and project period end dates ending the no-cost extension of this competitive segement due to the funding of 2 R56 AI 070908-06. The effective date for the R56 bridge award is also corrected in this term to 8/12/2013.

Supersedes Notice of Award (NoA) issued 08/09/2013.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Gregory P. Smith
Email: gsmith@niaid.nih.gov **Phone**: 301-402-5540 **Fax**: 301-493-0597

Program Official: Suman Mukhopadhyay
Email: mukhopadhyays@mail.nih.gov **Phone**: 301-451-3032 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI070908-05 REVISED

INSTITUTION: KANSAS STATE UNIVERSITY

Budget	***Year 5***
TOTAL FEDERAL DC	$245,025
TOTAL FEDERAL F&A	$112,712
TOTAL COST	$357,737

Facilities and Administrative Costs	***Year 5***
F&A Cost Rate 1	46%
F&A Cost Base 1	$245,025
F&A Costs 1	$112,712

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OMB No. 0925-0001

Grant Number R01AI070908-05	**Total Project Period** From: 12/15/2007 To: 11/30/2012
EIN: **Review Group:** 1480771751A1 ZRG1 IDM-B (02) M	**Requested Budget Period:** From: 12/01/2011 To: 11/30/2012
Title of Project: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression	**Due Date:** 10/16/2011 **Submitted Date:** 10/18/2011

Program Director/Principal Investigator: ROMAN R. GANTA Diagnostic Med./Pathobiology College of Veterinary Medicine 1800 Denison Ave Manhattan , KS 66506 **Phone Number:** 785-532-4612 **Fax Number:** 785-532-4039 **Email Address:** rganta@vet.k-state.edu	**Applicant Organization:** KANSAS STATE UNIVERSITY KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL MANHATTAN , KS 665061103 **Department:** DIAGNOSTIC MEDICINE PATHOBIOLOGY **Major Subdivision:** COLLEGE OF VETERINARY MEDICINE
Administrative Official: Carmen J Garcia KANSAS STATE UNIVERSITY 2 Fairchild Hall MANHATTAN , KS 665061103 **Phone Number:** 7855326804 **Fax Number:** 7855325944 **Email Address:** carmen@ksu.edu	**Signing Official:** Anita S Fahrny 2 Fairchild Hall Kansas State University Manhattan , KS 665061103 **Phone Number:** 785-532-6804 **Fax Number:** **Email Address:** afahrny@ksu.edu

Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ **Previously Reported** ☒ **Not Previously Reported**
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location

Organizational Name: Kansas State University		
DUNS: 929773554		
Street 1: Kansas State University	Street 2: 2 Fairchild Hall	
City: Manhattan	County:	State: KS
Province:	Country: UNITED STATES	Zip/Postal Code: 665061103
Congressional Districts: KS-002		

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Program Director/Principal Investigator:	**Grant Number**
ROMAN R. GANTA	R01AI070908-05
Applicant Organization:	**Period Covered by this Report:**
KANSAS STATE UNIVERSITY	12/01/2010 - 11/30/2011

Title of Project:

Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☐ **No** ☒ **Yes**

Justification: We anticipate that more than 25% of the funds from the current budget year will remain as a balance to be carried over to the next yearâs project period. This happened due to problems encountered at the beginning of this yearâs project while recruiting new scientists. Importantly, the delay in recruiting scientists resulted in the accumulation of unused funds. However, to compensate for the loss of time in recruiting, a decision was made to recruit scientists having considerably more research experience (and with a higher pay scale). One of the scientists hired on the project is employed as a research assistant professor. Therefore, it is anticipate that the unused funds of this year will be spent during the coming funding year because the personnel costs will be significantly more than originally budgeted on the project. Moreover, the costs for chemicals and supplies increased considerably in recent times. Considering these two important variables, we do not anticipate accumulation of any unused funds at the end of next budget period.

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID:	Citation ID:	Citation Source:	Citation Text:

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Cover Letter: File is not uploaded
Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

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All Personnel Report

Program Director/Principal Investigator:			Grant Number			
ROMAN R. GANTA			R01AI070908-05			
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
ROMAN R. GANTA	eRA Commons User Name	PHD, MS, BS, MOTH	Personal Info	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			Personal Info	EFFORT		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement		BS	XXX-XX-	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Project Manager (Microbiologist)				EFFORT		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement			XXX-XX-	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Scientist (Post-Doc)				EFFORT		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement		PHD	XXX-XX-	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Scientist (Post-Doc)				EFFORT		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Redacted by agreement	eRA Commons User Name		XXX-XX-	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Scientist (Res Asst Prof)				EFFORT		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			

Obtained via FOIA by White Coat Waste Project

Redacted by agreement				XXX-XX-	Months Devoted		
					to Project		
Role on Project:	**Supplement Support:**			**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Scientist (Post-Doc)					EFFORT		

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A. SPECIFIC AIMS: The central hypothesis of this study is that the transcription from the 28 kDa outer membrane protein (p28-Omp) multigene locus in *E. chaffeensis* is differentially regulated in response to environmental signals and that the host cell-specific gene expression is essential for the pathogen's survival in vertebrate and tick cell environments. Proposed **specific aims** are: **1)** Establish *in vitro* transcription system for mapping *Ehrlichia* promoters. **2)** Map transcriptional machinery in differentially expressed genes 14 and 19 of the p28-Omp locus. **3)** Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19. **4)** Evaluate knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific gene expression.

B. STUDIES AND RESULTS:

Specific Aim 1: Establish *in vitro* transcription system for mapping *Ehrlichia* promoters.

E. chaffeensis RNA polymerase purification: Considerable progress has been made in accomplishing the objectives of this aim. During the last couple of years, we established the method that was useful in successfully purifying native *E. chaffeensis* RNA polymerase. *E. chaffeensis* RNA polymerase was purified from the organisms grown in the canine macrophage cell line, DH82. Furthermore, we demonstrated its function to drive gene expression and also mapped the enzyme properties. The enzyme was active in driving gene expression of several gene promoters of *E. chaffeensis* as assessed by using *in vitro* transcription assays. The analysis included the promoter regions of p28-Omp 14 and p28-Omp 19 genes. We also recombinantly expressed the sigma 70 and sigma 32 subunits of *E. chaffeensis* RNA polymerase and used to enhance the function of native RNA polymerase. Furthermore, we demonstrated that *E. coli* RNA polymerase core enzyme combined with *E. chaffeensis* recombinant sigma factors can be used similar to *E. chaffeensis* native RNA polymerase. A manuscript describing the detailed characterization of *E. chaffeensis* RNA polymerase was published this year in the journal, BMC Microbiology. The purified enzyme is also a source for several ongoing experiments.

In vitro transcription: Considerable progress has already been made in establishing the *in vitro* transcription assays and was reported in the earlier progress reports. Work is ongoing in further evaluating mutational analysis in the promoter sequences of p28-Omp genes, p28-Omp 14 and 19 (described in detail below). Several additional genes were also assessed by *in vitro* transcription assays to identify transcriptionally active genes, which are recognized by RNA polymerase containing sigma 70 subunit or with alternate sigma factor (sigma 32). Most of these studies were performed using the plasmid constructs prepared using a G-less cassette containing plasmids. Unpublished describing the research results from the p28-Omp constructs with several promoter deletion segments Unpublished Unpublished

Specific Aim 2: Map transcriptional machinery in differentially expressed genes of the p28-Omp locus. We continue making considerable progress in support of this specific aim. This year, we created numerous point mutations in the -35 regions of p28-Omp 14 and 19 gene promoters as well as to create complete deletion mutations in this region. We generated considerable data useful in pinpointing the precise location of RNA polymerase binding regions for these two genes. The approaches we employed include creating overlapping point mutations, defining the locations of mutations by sequence analysis, and assess their impact on the gene function by measuring the β-galactosidase activity driven from the insertion of the enzyme coding sequence inserted downstream to promoter region. The analyses were performed in *E. coli* system that we developed as part of this year's research. This approach facilitated rapid assessment of numerous gene promoter mutations. Their validity will be further confirmed for a subset of mutations located within important domains of the promoter. We opted to do mutation analysis only in -35 region because our earlier analysis suggested that the loss of -10 region appeared to be not important for p28-Omp genes of *E. chaffeensis* (reported in one of our prior publications). This work is ongoing and we anticipate significant progress during the next year. This year we also initiated another approach to map the transcriptional machinery of *E. chaffeensis* where we planned to replace gene sequences of *E. coli* sigma 32 and 70 subunits with *E. chaffeensis* homologs. This novel approach will allow us to use *E. coli* system to study functions of various *E. chaffeensis* gene promoters. This is a challenging approach and although we made some progress in preparing the constructs, generation of functional complementation, gene exchange

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mutations with *E. chaffeensis* sigma 70 or sigma 32 gene segments in *E. coli* remains to be completed. We expect that this goal will be accomplished during this coming budget year. We also developed another approach where *E. chaffeensis* sigma factors are expressed extra-chromosomally in *E. coli* having mutations for its own sigma factors. We were successful in achieving this goal for sigma 32 subunit that was expressed in three different *E. coli* strains. We then used the recombinant strains to assess the functions of several *E. chaffeensis* genes, including a heat shock protein gene (GroES/L), DnaK, and DNA binding protein gene (HU). Using these new molecular tools, we have now initiated experiments to identify the DNA binding domains of promoters and sigma 32 subunit protein domains that interact with the promoter regions. These experiments will be part of the next 12 months of project period and these novel approaches will aid significantly in mapping the interactions of RNA polymerase with *E. chaffeensis* gene promoters. A post-doctoral scientist and another scientist hired as a research assistant professor have been active in accomplishing the goals of this aim.

Specific Aim 3: Evaluate the contributions of macrophages and tick cell environments for differential expression from genes 14 and 19 of the p28-Omp locus: During previous funded project years, we established several methods needed for recovering purified *E. chaffeensis* proteins and to assess interactions between DNA and DNA binding proteins. A post-doctoral scientist was appointed this year to focus more on this area of research. Although the influence of *E. chaffeensis* protein extracts has already been tested and the data were incorporated in the manuscript published this year, more work remains to be performed in establishing the identity of the proteins. As we reported earlier, we already recombinantly expressed several putative regulatory proteins of *E. chaffeensis*. The recombinant proteins in purified form are now available and they will be used to assess if they bind to p28Omp 14 and 19 promoters. These analyses will be performed initially by using gel shift assays, and then by performing affinity purification methods to map direct interactions. Furthermore, experiments will be performed to isolate Ehrlichia proteins that control the gene expression from p28-Omp 14 and 19 genes that are host cell-specifically expressed. This part of the project is ongoing and we anticipate making significant progress during the next 12 months of budget period.

Specific Aim 4: Evaluation of knockout mutations for p28-Omp genes 14 and 19 to assess the biological relevance of host cell specific gene expression. The goal of this aim is to develop knockout systems useful in testing the hypothesis that unique host cell-specific gene expression is essential for the pathogen's survival in vertebrate and tick cell environments. This goal remained as the major challenge for the project. We identified numerous targets to create mutations that do not lead to detrimental outcomes for the *E. chaffeensis* survival. This way, we will be able to develop protocols for mutational generation. Earlier, we reported the preparation of numerous recombinant plasmid constructs to create mutations. We also reported our continued efforts in identifying viable mutations. The approaches we employed included homologous recombination and modified group II intron-based mutagenesis strategy (Targetron mutagenesis) for targeting to specific genomic regions. In addition, this year we included transposon-based mutagenesis methods. The constructs we prepared included six genomic regions, one of them targeting to Ech_0126 gene and others include targets to p28-Omp genes. Both methods yielded several detectible viable mutants (judged by PCR, Southern blot analysis and sequence analysis). However, viable mutants could not be purified or recovered beyond 8 days post-insertion of mutations. These data may indicate that the regions we selected may be critical for the *Ehrlichia* survival or alternatively mutants may grow at a slower rate. Our efforts to clone using antibiotic selection strategy were unsuccessful, possibly because the antibiotic resistance protein expression may not be optimal for the bacterium. Therefore, we have been employing alternate means of selecting the mutants, i.e., by performing serial dilution experiments. Progress from this work was presented at the recent 6th International meeting on Rickettsiae and Rickettsial Diseases held in Hearaklyon, Greece (June 5-7, 2011) (this meeting was held in collaboration with scientists of the American Society for Rickettsiology). These studies will continue as part of the next year's planned research.

C. SIGNIFICANCE: Progress from this year's study included the establishment of methods to purify native and recombinant *E. chaffeensis* RNA polymerase and their use in mapping the bacterial genes under *in vitro* conditions. The work was reported in a peer-reviewed scientific journal and the manuscript was accessed by large number of scientists (we received a response survey from the journal stating that the article was accessed 847 times as of September 5, 2011 (that is in about 5 months after it first appeared

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online). The project progress also included establishing methods to map promoters of *E. chaffeensis*. These studies are important not only for the understanding the pathogenicity caused by *E. chaffeensis*, but also for other tick-borne rickettsial organisms responsible for significant health impact on people and animals. This project is the first to develop many new methods and the studies will be important in leading the way for parallel research in other important related tick-borne rickettsial pathogens.

D. PLANS: We will continue our work on aims 2-4 (goals of aim 1 were complete) of the proposal with a plan to complete the project goals. This year, we will focus more on defining the promoter regions, contributions of domains of RNA polymerase that interact with promoters, identifying the regulatory proteins involved in differential gene regulation. We also will conduct experiments to further assess the feasibility in identifying the viable mutants of *E. chaffeensis*.

E. PUBLICATIONS (published or submitted):

Isolation and characterization of Ehrlichia chaffeensis RNA polymerase and its use in evaluating p28 outer membrane protein gene promoters. Bonto Faburay , Huitao Liu , Lalitha Peddireddi and Roman R Ganta BMC Microbiology 2011, 11:83doi:10.1186/1471-2180-11-83 [PMID:21513529, PMCID: PMC3108270]

Unpublished

F. ABSTRACTS:

Mutational analysis of Ehrlichia chaffeensis. Roman R. Ganta. The 6th International meeting on Rickettsiae and Rickettsial Diseases, Heraklion, Crete, Greece, 5-7 June 2011.

Obtained via FOIA by White Coat Waste Project

Grant Number: 2R56AI070908-06 REVISED

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Mr. Lowe, Paul R.
Asst Vice President/Research
2 Fairchild Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Budget Period: 08/12/2013 – 07/31/2014
Project Period: 12/15/2007 – 07/31/2014

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R56AI070908. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Gregory P. Smith
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Obtained via FOIA by White Coat Waste Project

Additional information follows

Obtained via FOIA by White Coat Waste Project

NIH NGA R | Version: 431 - 02/06/2013 13:43:00 | Generated on: 08/22/2013 00:07:19

SECTION I – AWARD DATA – 2R56AI070908-06 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$133,198
Fringe Benefits	$37,602
Personnel Costs (Subtotal)	$170,800
Supplies	$55,278
Travel Costs	$9,213
Other Costs	$4,246
Consortium/Contractual Cost	$53,729
Federal Direct Costs	$293,266
Federal F&A Costs	$132,269
Approved Budget	$425,535
Federal Share	$425,535
TOTAL FEDERAL AWARD AMOUNT	$425,535
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
6	$425,535	$425,535

Fiscal Information:

CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908B
Fiscal Year:	2013

IC	*CAN*	*2013*
AI	8480847	$425,535

NIH Administrative Data:
PCC: M46C / **OC**: 414B / **Released**: eRA Commons 08/21/2013
Award Processed: 08/22/2013 12:07:19 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 2R56AI070908-06 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 2R56AI070908-06 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

Obtained via FOIA by White Coat Waste Project

This grant is excluded from Streamlined Noncompeting Award Procedures (SNAP).
This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/#gps .

A final Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 90 days of the expiration date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) cash transaction data.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 90 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

Unless an application for competitive renewal is submitted, a final progress report must also be submitted within 90 days of the expiration date. Instructions for preparing a Final Progress Report are at: http://grants.nih.gov/grants/funding/finalprogressreport.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report. Institute/Centers may accept the progress report contained in competitive renewal (type 2) in lieu of a separate final progress report. Contact the awarding IC for IC-specific policy regarding acceptance of a progress report contained in a competitive renewal application in lieu of a separate final progress report.

NIH **strongly encourages** electronic submission of the final progress report and the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final progress report and final invention statement may be e-mailed as PDF attachments to the NIH Central Closeout Center at: DeasCentralized@od.nih.gov.

Hard copy: Paper submissions of the final progress report and the final invention statement may be faxed to the NIH Division of Central Grants Processing at 301-480-2304, or mailed to:

NIH Division of Central Grants Processing, OER
6705 Rockledge Drive
Suite 5016, Room 5109
MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final Progress Report is not required. However, a final FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

Obtained via FOIA by White Coat Waste Project

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 2R56AI070908-06 REVISED

This award was revised to correct the Project Period Start Date.

Supersedes Notice of Award (NOA) issued 08/09/2013.
*****

This award is issued in accordance with FY2013 NIH Fiscal Policy per NOT-OD-13-064, http://grants.nih.gov/grants/guide/notice-files/NOT-OD-13-064.html .
~~~~~~~

This award provides interim funding for a period of one year at a level not to exceed 20% over the direct costs awarded for the last non-competing continuation. If an amended application for research project 2 R01 AI070908-06 is funded prior to the termination of this project period, this award will be adjusted accordingly
~~~~~~~

This award includes funds awarded for consortium activity with Oklahoma State University in the amount of $53,729 ($36,851 direct costs + $16,878 facilities and administrative costs). Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2012/nihgps_ch15.htm#_Toc271265264

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Gregory P. Smith
Email: gsmith@niaid.nih.gov **Phone**: 301-402-5540 **Fax**: 301-493-0597

Program Official: Suman Mukhopadhyay
Email: mukhopadhyays@mail.nih.gov **Phone**: 301-451-3032 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 2R56AI070908-06 REVISED

INSTITUTION: KANSAS STATE UNIVERSITY

Budget	***Year 6***
Salaries and Wages	$133,198
Fringe Benefits	$37,602
Personnel Costs (Subtotal)	$170,800
Supplies	$55,278
Travel Costs	$9,213
Other Costs	$4,246
Consortium/Contractual Cost	$53,729
TOTAL FEDERAL DC	$293,266
TOTAL FEDERAL F&A	$132,269
TOTAL COST	$425,535

Facilities and Administrative Costs	***Year 6***
F&A Cost Rate 1	50%

Obtained via FOIA by White Coat Waste Project

F&A Cost Base 1	$264,537
F&A Costs 1	$132,269

Obtained via FOIA by White Coat Waste Project

Notice of Award

RESEARCH **Issue Date:** 07/31/2014

Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 2R01AI070908-06A1
FAIN: R01AI070908

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Mr. Lowe, Paul R.
Asst Vice President/Research
2 Fairchild Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Budget Period: 08/01/2014 – 07/31/2015
Project Period: 08/01/2006 – 07/31/2018

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $441,044 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI070908. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Gregory P. Smith

Obtained via FOIA by White Coat Waste Project

Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Obtained via FOIA by White Coat Waste Project

SECTION I – AWARD DATA – 2R01AI070908-06A1

Award Calculation (U.S. Dollars)

Salaries and Wages	$139,958
Fringe Benefits	$40,858
Supplies	$87,425
Travel Costs	$9,801
Other Costs	$15,987
Federal Direct Costs	$294,029
Federal F&A Costs	$147,015
Approved Budget	$441,044
Federal Share	$441,044
TOTAL FEDERAL AWARD AMOUNT	$441,044
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$441,044

SUMMARY TOTAL FEDERAL AWARD AMOUNT YEAR (6)	
GRANT NUMBER	**TOTAL FEDERAL AWARD AMOUNT**
2R01AI070908-06A1	$441,044
2R56AI070908-06	$425,535
TOTAL	**$866,579**

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
6	$441,044	$866,579
7	$441,044	$441,044
8	$441,044	$441,044
9	$441,044	$441,044

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908C
PMS Account Type:	P (Subaccount)
Fiscal Year:	2014

IC	CAN	2014	2015	2016	2017
AI	8472302	$441,044	$441,044	$441,044	$441,044

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C / **OC**: 414B / **Released** eRA Commons User Name 07/25/2014
Award Processed: 05/08/2014 01:52:21 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 2R01AI070908-06A1

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 2R01AI070908-06A1

Obtained via FOIA by White Coat Waste Project

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI070908. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 2R01AI070908-06A1

Direct costs awarded are limited to a 20% increase over the direct costs awarded in the last non-competing continuation (see NIH Guide, Vol. 26, No. 35, 10/17/97).

*****

To meet Institute program objectives, this award is adjusted to 4 years of funding.

*****

NIAID Grants staff will be moving to a new building effective October 14, 2014. As a result of the move the telephone numbers listed on this NoA for your grants management specialist and program officers may no longer be active. Please check the website below to find their new telephone numbers:

https://ned.nih.gov/search/

Obtained via FOIA by White Coat Waste Project

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Gregory P. Smith
Email: gsmith@niaid.nih.gov **Phone**: 301-402-5540 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 240-627-3341 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 2R01AI070908-06A1

INSTITUTION: KANSAS STATE UNIVERSITY

Budget	Year 6	Year 7	Year 8	Year 9
Salaries and Wages	$139,958	$139,958	$139,958	$139,958
Fringe Benefits	$40,858	$40,858	$40,858	$40,858
Supplies	$87,425	$87,425	$87,425	$87,425
Travel Costs	$9,801	$9,801	$9,801	$9,801
Other Costs	$15,987	$15,987	$15,987	$15,987
TOTAL FEDERAL DC	$294,029	$294,029	$294,029	$294,029
TOTAL FEDERAL F&A	$147,015	$147,015	$147,015	$147,015
TOTAL COST	$441,044	$441,044	$441,044	$441,044

Facilities and Administrative Costs	Year 6	Year 7	Year 8	Year 9
F&A Cost Rate 1	50%	50%	50%	50%
F&A Cost Base 1	$294,029	$294,029	$294,029	$294,029
F&A Costs 1	$147,015	$147,015	$147,015	$147,015

Obtained via FOIA by White Coat Waste Project

PI: **GANTA, ROMAN R.**	Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression	
Received: 07/19/2013	FOA: PA11-260	Council: 01/2014
Competition ID: ADOBE-FORMS-B2	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
2 R01 AI070908-06A1	Dual:	Accession Number: 3611148
IPF: 4202801	Organization: KANSAS STATE UNIVERSITY	
Former Number:	Department: Diagnostic Medicne Pathobiolog	
IRG/SRG: ZRG1 IDM-P (02)S	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 6: 300,000 Year 7: 300,000 Year 8: 300,000 Year 9: 300,000 Year 10: 300,000	Animals: Y Humans: N Clinical Trial: N Current HS Code Evaluative Info HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Roman Ganta	Kansas State University	PD/PI
Redacted by agreement		Other (Specify)-Co-Investigator
		Consultant

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 06/30/2011

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION
☐ Pre-application ☒ Application ☐ Changed/Corrected Application

2. DATE SUBMITTED: 07/19/2013
Applicant Identifier:

4. a. Federal Identifier: AI070908
b. Agency Routing Identifier:

5. APPLICANT INFORMATION
*** Organizational DUNS:** 929773554

* Legal Name: Kansas State University
Department: | Division:
* Street1: 2 Fairchild Hall
Street2:
* City: Manhattan | County / Parish: Riley
* State: KS: Kansas | Province:
* Country: USA: UNITED STATES | * ZIP / Postal Code: 66506-1103

Person to be contacted on matters involving this application
Prefix: Mr. | * First Name: Paul | Middle Name: R.
* Last Name: Lowe | Suffix:
* Phone Number: 785-532-6804 | Fax Number: 785-532-5944
Email: plowe@ksu.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN)*: 1-480771751-A1

7. * TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education
Other (Specify):
Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:
☐ New ☒ Resubmission
☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).
☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration
☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

12. PROPOSED PROJECT:
* Start Date: 04/01/2014 | * Ending Date: 03/31/2019

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT**: KS-001

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION
Prefix: Dr. | * First Name: Roman | Middle Name: Reddy
* Last Name: Ganta | Suffix:
Position/Title: Professor
* Organization Name: Kansas State University
Department: Diagnostic Medicne Pathobiolog | Division: Veterinary Medical Center
* Street1: 310 Mosier KSU
Street2:
* City: Manhattan | County / Parish: Riley
* State: KS: Kansas | Province:
* Country: USA: UNITED STATES | * ZIP / Postal Code: 66506-5601
* Phone Number: 785/532-4612 | Fax Number: 785532-3939
* Email: rganta@ksu.edu

Obtained via FOIA by White Coat Waste Project

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	2,250,000.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	2,250,000.00
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: Mr. * First Name: Paul Middle Name: R.

* Last Name: Lowe Suffix:

* Position/Title: Asst Vice President/Research

* Organization: Kansas State University

Department: PreAward Services Division: Research & Sponsored Programs

* Street1: 2 Fairchild Hall

Street2:

* City: Manhattan County / Parish: Riley

* State: KS: Kansas Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 66506-1103

* Phone Number: 785-532-6804 Fax Number: 785-532-5944

* Email: plowe@ksu.edu

*** Signature of Authorized Representative**: Paul R. Lowe

*** Date Signed**: 07/19/2013

20. Pre-application Add Attachment | Delete Attachment | View Attachment

Obtained via FOIA by White Coat Waste Project

424 R&R and PHS-398 Specific Table Of Contents

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Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0010
Expiration Date: 08/31/2011

Project/Performance Site Location(s)

Project/Performance Site Primary Location ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Kansas State University

DUNS Number: 9297735540000

* Street1: 2 Fairchild Hall

Street2:

* City: Manhattan County: Riley

* State: KS: Kansas

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 66506-1103 * Project/ Performance Site Congressional District: KS-001

Project/Performance Site Location 1 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City: County:

* State:

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: * Project/ Performance Site Congressional District:

Additional Location(s) Add Attachment Delete Attachment View Attachment

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED Other Project Information

OMB Number: 4040-0001

1. Are Human Subjects Involved? ☐ Yes ☒ No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number: A3609-01

3. Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. Does this Project Have an Actual or Potential Impact - positive or negative - on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. Project Summary/Abstract ProjectSummary1008827004.pdf Add Attachment | Delete Attachment | View Attachment

8. Project Narrative ProjectNarrative1008826983.pdf Add Attachment | Delete Attachment | View Attachment

9. Bibliography & References Cited References1008827000.pdf Add Attachment | Delete Attachment | View Attachment

10. Facilities & Other Resources FacilitiesOtherResources1008827003.pd Add Attachment | Delete Attachment | View Attachment

11. Equipment Equipment1008826984.pdf Add Attachment | Delete Attachment | View Attachment

12. Other Attachments Add Attachments | Delete Attachments | View Attachments ☐

Obtained via FOIA by White Coat Waste Project

PROJECT SUMMARY

Illnesses caused by rickettsiales of the genera *Ehrlichia* and *Anaplasma* are a growing human health concern in recent years and are the second leading cause of tick-borne infections in the USA and many parts of the world. They include human monocytic ehrlichiosis caused by *Ehrlichia chaffeensis*. Despite the sophisticated systems of defense in vertebrate and tick hosts, the rickettsiales evade the host clearance. *E. chaffeensis* in macrophages and tick cells differ significantly in expression of several outer membrane proteins and effector proteins secreted via its secretary pathways. Research results demonstrate that differential tick and vertebrate host cell-specific expression contributes to the varied host response and delayed clearance in a host. The central hypothesis of the previously funded project is that *E. chaffeensis* differentially regulates gene expression and that the host-specific gene expression is essential for its survival in vertebrate and tick cells. Progress from the previous funding cycle forms the strong foundation for this renewal application and to continue testing the above stated innovative hypothesis. Specific aims of this application are 1) to characterize *E. chaffeensis* RNA polymerase complex in support of understanding how vector and vertebrate host-specific differential gene expression is accomplished, 2) to evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth, and 3) to perform mutational analysis and *in vivo* screening to identify genes essential for the *E. chaffeensis* pathogenesis.

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PROJECT NARRATIVE

The results from this study will provide important information for understanding *E. chaffeensis* pathogenesis, gene regulation and how the rickettsiale in vertebrate and tick hosts respond to the loss of expression from differentially expressed genes. This study will also allow us to determine how the tick transmitted pathogen persists and will aid in identifying targets for controlling *E. chaffeensis* infections.

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FACILITIES & OTHER RESOURCES

Laboratories: Dr. Ganta has three well-equipped research laboratories (450 sq. ft. each). Two laboratories have been designated for use in studies involving cell culture, nucleic acid and protein isolation and for setting up in vitro amplification procedures. The third laboratory is for conducting experiments where PCR product analysis, molecular cloning and protein analysis experiments, immunoblot and ELISA analysis are performed. Each laboratory has its own dedicated equipment items. Chemicals, necessary reagents and consumables are maintained separately in each laboratory.

Offices: We have three 150 sq. ft. offices for use by the research personnel. The department has a well-organized office constituting several secretarial staff and accountants to support the project needs.

Computers: Several personal computers are installed in all of the offices and in laboratories. All of the computers have Ethernet connections for the direct high speed access. The facilities also include high-speed wireless access for Internet browsing. We have a lifetime license for nucleic acid analysis software. Our college maintains a multimedia center and a technical support center.

Major Equipment in my laboratory: The equipment in Dr. Ganta's laboratories include two electroporators, two conventional 96 tube capacity PCR machines (a Perkin-Elmer GenAmp 9700 and an Eppindorf temperature gradient PCR with an in situ PCR module), a Smart Cycler PCR system and a BioRad iCycler system for real time PCR/RT-PCR analysis, manual and automated DNA sequencing systems, several agarose and poly acrylamide gel systems, a pulsed field gel apparatus, a 2D gel electrophoresis system for protein, DNA and RNA analysis, a hybridization oven, a blot transfer apparatus, a UV cross linker, Kodak image analysis system, an X-ray film developer, several water baths, shaker incubators, a speed-vac system, a sequencing gel drier system, several micro centrifuges, a sonicator, four CO2 incubators, two sterile cell-culture hoods, two cyto-centrifuges, a sero-centrifuge, three fume hoods, a cryogenic freezer, three chromatography refrigerators, three freezers, several liquid nitrogen tanks, several weighing balances (micro and macro), three refrigerated high speed centrifuges, and pH meters. We are also equipped with a fluorescence microscope with the capability to record digital images.

We also have the complete access to many shared facilities. They include several additional PCR machines, X-ray film developers, ice machines, scintillation counters, autoclaves, and a centralized deionized water supply. We also have a walk-in cold room, a 37 degree incubator, and an instrumentation room containing several pieces of commonly used equipment such as the high speed and ultra centrifuges. These are located within the close proximity of my laboratories. Our department also has a transmission and scanning electron microscope, a Becton Dickinson FACScan for flow cytometry analysis and maintains a core facility for confocal miscroscopic facility.

Kansas State University has several biotechnology core facilities to support research on genomics, functional genomics and molecular biology. The facilities include several state-of-the-art pieces of equipment, such as the MALDI-TOF and LC-MS/MS mass spectrometry instruments and protein/peptide sequencing equipment (http://www-personal.ksu.edu/~jtomich/corelab.html).

Obtained via FOIA by White Coat Waste Project

EQUIPMENT

The equipment in Dr. Ganta's laboratories includes two electroporators, two conventional 96 tube capacity PCR machines (a Perkin-Elmer GenAmp 9700 and an Eppindorf temperature gradient PCR with an in situ PCR module), a Smart Cycler PCR system and a BioRad iCycler system for real time PCR/RT-PCR analysis, manual and automated DNA sequencing systems, several agarose and poly acrylamide gel systems, a pulsed field gel apparatus, a 2D gel electrophoresis system for protein, DNA and RNA analysis, a hybridization oven, a blot transfer apparatus, a UV cross linker, Kodak image analysis system, an X-ray film developer, several water baths, shaker incubators, a speed-vac system, a sequencing gel drier system, several micro centrifuges, a sonicator, four CO2 incubators, two sterile cell-culture hoods, two cyto-centrifuges, a sero-centrifuge, three fume hoods, a cryogenic freezer, three chromatography refrigerators, three freezers, several liquid nitrogen tanks, several weighing balances (micro and macro), three refrigerated high speed centrifuges, and pH meters. We are also equipped with a fluorescence microscope with the capability to record digital images.

We also have the complete access to many shared facilities. They include several additional PCR machines, X-ray film developers, ice machines, scintillation counters, autoclaves, and a centralized deionized water supply. We also have a walk-in cold room, a 37 degree incubator, and an instrumentation room containing several pieces of commonly used equipment such as the high speed and ultra centrifuges. These are located within the close proximity of my laboratories. Our department also has a transmission and scanning electron microscope, a Becton Dickinson FACScan for flow cytometry analysis and maintains a core facility for confocal miscroscopic facility.

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix:	Dr.	* First Name: Roman	Middle Name: Reddy
* Last Name:	Ganta	Suffix:	
Position/Title:	Professor	Department:	Diagnostic Medicne Pathobiolog
Organization Name:	Kansas State University	Division:	Veterinary Medical Center
* Street1:	310 Mosier KSU		
Street2:			
* City:	Manhattan	County/ Parish:	Riley
* State:	KS: Kansas	Province:	
* Country:	USA: UNITED STATES	* Zip / Postal Code:	66506-5601
* Phone Number:	785/532-4612	Fax Number:	785532-3939
* E-Mail:	rganta@ksu.edu		

Credential, e.g., agency login: eRA Commons User Name

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: PhD

Degree Year: 1987

***Attach Biographical Sketch**	BiographicalSketch_Ganta10088	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment

PROFILE - Senior/Key Person 1

Redacted by agreement

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

Redacted by agreement

Obtained via FOIA by White Coat Waste Project

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Roman R. Ganta	**Professor**
eRA COMMONS USER NAME eRA Commons User Name	

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Andhra University, Waltair, India	B. S.	1975-78	Zoology, Botony, Chemistry
Andhra University, Waltair, India	M.S.	1978-80	Biochemistry
All India Institute of Medical Sciences, New Delhi, India	Ph.D.	1982-87	Biochemistry
The Scripps Research Institute, La Jolla, CA	Postdoctoral	1987-90	Molecular Biology
University of Florida, Gainesville, FL	Postdoctoral	1990-92	Molecular Parasitology

A. PERSONAL STATEMENT: Dr. Ganta has 22 years focused on research related to important human and animal infectious diseases. His primary research focus for over 18 years has been on *Ehrlichia/Anaplasma* species pathogens. His studies involve assessing pathogen molecular structure, host responses, and the effect of vector and host cell environments on the pathogens' adaptation. His research experiences include the development of molecular diagnostic tests, molecular cloning and evaluation of genes and their gene products, genome and expressed sequence tag analysis, proteomics and microarray analysis, immunology, evaluating the genes and animal infection studies and to understand the host-pathogen interactions involving the pathogen, host and vector. He built a strong research team of scientists and collaborators who have been actively engaged in accomplish the objectives of the research executed and proposed efficiently and in a timely manner. In particular, his research remained focused in how *Ehrlichia chaffeensis* overcomes the host stress during its growth in vertebrate and tick hosts. The collaborative research led to many novel findings regarding the pathogen's growth in vertebrate and tick host cell environments. The project goals for his competing renewal application are in line with his high level expertize in *Ehrlichia* molecular biology and animal infection studies.

B. POSITIONS AND HONORS.

Positions and Employment

1992-98 Assistant Scientist, Dept. Pathobiology, University of Florida, Gainesville, FL.
1998-03 Assistant Professor, Department of Diagnostic Medicine/Pathobiology, Kansas State University.
2003-2007 Associate Professor, Department of Diagnostic Medicine/Pathobiology, Kansas State University.
2007- Professor, Department of Diagnostic Medicine/Pathobiology, Kansas State University.

Other Experience and Professional Memberships

2003 Ad-hoc member, Bacteriology and Mycology 1 (BM1) Study Section, NIH
2004 Ad-hoc member, Host Interaction with Bacterial Pathogens (HIBP) Study Section, NIH
2005, 09-13 Ad-hoc member, Infectious Diseases and Microbiology (IDM) Study Section, NIH
2006-10 Member of the Vector Biology (VB) Study Section, NIH
2002-12 Oklahoma Center for the Advancement of Science and Technology, Oklahoma City, OK Grants Review panel
2005-current Director, Molecular Biology Core, Center Of Biomedical Research Excellence (COBRE) Epithelial Function in Health and Disease at CVM, KSU
1998-current Member, American Society for Rickettsiology
2003-07 Secretary/Treasurer, American Society for Rickettsiology
2007-09 Vice-President, American Society for Rickettsiology
2009-10 President, American Society for Rickettsiology
2001-current Member, American Society for Microbiology
2008-current Council Member (20010-current) and Vector-borne and Parasitic diseases Section Leader (2008-current), Conference of Research Workers in Animal Diseases (CRWAD), USA

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Honors

Award:

2005 Pfizer Animal Health Award for Research Excellence, College of Veterinary Medicine, KSU

Editorial Boards:

2009-current Editorial Board: Current Trends in Biotechnology and Pharmacy (ISSN 0973-8916)
2010-current Regional Editor: Research Journal of Microbiology (ISSN: 1816-4935)
2011-current Editorial Board member, Frontiers in Microbiology
2011-current Academic Editor, PLoS ONE

C. PEER-REVIEWED PUBLICATIONS MOST RELEVANT TO CURRENT APPLICATION (Selected 15 from over 55 peer-reviewed publications. Note: Name in some papers appears as G. R. Reddy):

1. Aggregate-Reactivation Activity of the Molecular Chaperone ClpB from Ehrlichia chaffeensis. Zhang T, Kedzierska-Mieszkowska S, Liu H, Cheng C, **Ganta RR**, Zolkiewski M. PLoS One. 2013 May 7;8(5):e62454. doi: 10.1371/journal.pone.0062454. PMID: 23667479 PMCID: PMC3646808

2. Targeted and Random Mutagenesis of *Ehrlichia chaffeensis* for the Identification of Genes Required for In vivo Infection. Cheng C, Nair ADS, Indukuri VV, Gong S, Felsheim RF, Jaworski D, Munderloh UG and **Ganta RR**. PLoS Pathog. 2013 Feb;9(2):e1003171. doi: 10.1371/journal.ppat.1003171. Epub 2013 Feb 14. PMID: 23459099 PMCID: PMC3573109

3. Dedonder SE, Cheng C, Willard LH, Boyle DL, **Ganta RR**. Transmission electron microscopy reveals distinct macrophage- and tick cell-specific morphological stages of Ehrlichia chaffeensis. PLoS One. 2012;7(5):e36749. Epub 2012 May 15. PMID: 22615806 PMCID: PMC3352939

4. Identification of critical host mitochondrion-associated genes during Ehrlichia chaffeensis infections. Von Ohlen T, Luce-Fedrow A, Ortega MT, **Ganta RR**, Chapes SK. Infect Immun. 2012 Oct;80(10):3576-86. Epub 2012 Jul 30. PMID: 22851751 PMCID: PMC3457586

5. Faburay B, Liu H, Peddireddi L, **Ganta RR**. Isolation and characterization of Ehrlichia chaffeensis RNA polymerase and its use in evaluating p28 outer membrane protein gene promoters. BMC Microbiol. 2011, 22;11:83. PMCID: PMC3108270

6. Peddireddi L., Cheng C. and **Ganta R. R.** Promoter Analysis of Macrophage- and Tick Cell-specific Differentially Expressed *Ehrlichia chaffeensis* p28-Omp Genes *BMC Microbiology* 2009, 9:99. PMCID: PMC2694197

7. Seo G., Cheng C., Tomich J. and **Ganta R. R.** Total, membrane, and immunogenic proteomes of macrophage- and tick cell-derived *Ehrlichia chaffeensis* evaluated by LC-MS/MS and MALDI-TOF methods. *Infect Immun.* 2008 76:4823-4832. PMCID: PMC2573352

8. **Ganta R. R.,** Cheng C., Miller E. C., McGuire B. L., Peddireddi L., Sirigireddy K. R. and Chapes S. K.. Differential clearance and immune responses to tick cell vs. macrophage culture-derived *Ehrlichia chaffeensis* in mice. *Infect Immun*. 2007 Jan;75:135-45. Epub 2006 Oct 23.

9. Singu V., Peddireddi L., Sirigireddy K. R., Cheng C. and **Ganta R. R.** Unique microphage and tick cell-specific protein expression from the p28/p30-outer membrane protein multigene locus in Ehrlichia chaffeensis and Ehrlichia canis. *Cell. Microbiol.* 8:1475-1487 (2006).

10. Sirigireddy K. R. and **Ganta R. R**. Multiplex Detection of Ehrlichia and Anaplasma species pathogens in Peripheral Blood by Real-Time RT PCR. *The Journal of Molecular Diagnostics*., 7, 308-316 (2005).

11. Singu, v., Liu H., Cheng C. and **Ganta R. R.** *Ehrlichia chaffeensis* expresses macrophage and tick cell-specific 28 kDa outer membrane proteins. *Infect. Immun*., 73, 79-87 (2005)

12. **Ganta R. R.,** Cheng C., Wilkerson M. J. and Chapes S. K. Delayed clearance of *Ehrlichia chaffeensis* infection in CD4+ T-cell knockout mice. *Infect. Immun.* 72:159-167 (2004).

13. Cheng C., Paddock C. D., and **Ganta R. R.** Molecular heterogeneity of *Ehrlichia chaffeensis* isolates as determined by the sequence analysis of 28 kDa outer membrane protein genes and other regions of the genome. *Infect. Immun.,* 71, 187-195, (2003).

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14. **Ganta R. R**., Wilkerson M., Cheng C., Rokey A., and Chapes S. K.. Persistent *Ehrlichia chaffeensis* infection occurs in the absence of functional MHCII genes. *Infect Immun*., 70, 380-388, (2002).

15. **Reddy G. R.,** Sulsona, C. R., Barbet A. F., Mahan S. M., Burridge M. J. and Alleman A. R. Molecular characterization of a 28 kDa surface antigen gene family of the tribe Ehrlichiae. *Biochem. Biophy. Res. Commun.,* 247, 636-643 (1998).

D. RESEARCH SUPPORT:

ACTIVE

R01 AI070908A (Ganta R) 12/15/07-11/30/13 (with one year no cost extension)
NIH NIAID
Vector and host contributions to the regulation of *E. chaffeensis* gene expression

The major goals of this project are to evaluate the contributions of vector and host cell environment in altering the pathogen gene expression.

Private Source (Ganta R) 09/01/12-08/31/13
Clinical and diagnostic assessment of Ehrlichia and Anaplasma species infections in dogs

The major goal of this project is to conduct experimental infections in dogs with five closely related rickettsial pathogens; *Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia ewingii, Anaplasma phagocytophilum* and *Anaplasma platys*.

1P30 GM103326-01A1 (Govind R) 12/03/12-07/31/13
University of Kansas Medical Center Research Institute
Role of TcdR, the alternate sigma factor in Clostridium difficile virulence
Role on the project for Ganta: Mentor for KSU Subaward PI, Govind.

8 P20 GM103418-12 (Govind, R) 05/01/13-04/30/14
University of Kansas Medical Center Research Institute
MSG Mentor – Roman Ganta

The major goal of this project is to determine the toxin secretion mechanism in *Clostridium difficile*
Role on the project for Ganta: Mentor for KSU Subaward PI, Govind.

Completed

NIH-R13: AI084375-01 08/15/09 – 07/31/10
Title: American Society for Rickettsiology conference 2009. The goals of this project are to support the ASR conference to be held in Hilton Head Island, South Carolina, from August 15-18, 2009.
PI: R.R. Ganta

P20 RR17686 (Marcus D) 07/01/05-06/30/13
NIH NCRR
Epithelial function in health and disease

The goal of this project is to establish a Center of Excellence for the investigation of the function of epithelial cells. The primary purpose of the Center will be to develop the research careers of basic scientists and clinician scientists at the three institutions.
Role on the project for Ganta: Director for the Molecular Biology Support Core.

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Page 155 of 430 to Page 162 of 430

Withheld pursuant to exemption

Redacted by agreement

of the Freedom of Information and Privacy Act

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 9297735540000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry *** Start Date:** 04/01/2014 *** End Date:** 03/31/2015 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Base Salary	EFFORT			0.00	0.00	0.00
2.	Dr.	Redacted by agreement				Co-Investigator					42,000.00	14,280.00	56,280.00
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 56,280.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

	* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	2	Post Doctoral Associates	EFFORT			80,000.00	27,200.00	107,200.00
		Graduate Students						
	2	Undergraduate Students	12.00			20,800.00	208.00	21,008.00
		Secretarial/Clerical						
	1	Microbiologist	EFFORT			0.00	0.00	0.00
	5	**Total Number Other Personnel**					**Total Other Personnel**	128,208.00

Total Salary, Wages and Fringe Benefits (A+B) 184,488.00

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2014 * End Date: 03/31/2015 Budget Period 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
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5.		
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8.		
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10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	10,000.00
2.	Foreign Travel Costs	
	Total Travel Cost	10,000.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2014 * End Date: 03/31/2015 Budget Period 1

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	89,200.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal Per Diem	10,920.00
9.	Blood Collection/Sample Processing	5,392.00
10.		
	Total Other Direct Costs	105,512.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	300,000.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	On campus Organized Research MTDC r	50.00	300,000.00	150,000.00
2.				
3.				
4.				
	Total Indirect Costs			150,000.00

Cognizant Federal Agency US Department of Health and Human Services, Arif Karim, 214-76

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	450,000.00

J. Fee

	Funds Requested ($)

K. * Budget Justification BudgetJustification1008827007.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 9297735540000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry *** Start Date:** 04/01/2015 *** End Date:** 03/31/2016 **Budget Period** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Base Salary	EFFORT			0.00	0.00	0.00
2.	Dr.	Redacted by agreement				Co-Investigator					42,000.00	14,280.00	56,280.00
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 56,280.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
2	Post Doctoral Associates	EFFORT			80,000.00	27,200.00	107,200.00
	Graduate Students						
2	Undergraduate Students	12.00			20,800.00	208.00	21,008.00
	Secretarial/Clerical						
1	Microbiologist	EFFORT			0.00	0.00	0.00
5	**Total Number Other Personnel**					**Total Other Personnel**	128,208.00

Total Salary, Wages and Fringe Benefits (A+B) 184,488.00

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2015 * End Date: 03/31/2016 Budget Period 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
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9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	10,000.00
2.	Foreign Travel Costs	
	Total Travel Cost	10,000.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2

Next Period

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2015 * End Date: 03/31/2016 Budget Period 2

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	89,200.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal Per Diem	10,920.00
9.	Blood Collection/Sample Processing	5,392.00
10.		
	Total Other Direct Costs	105,512.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	300,000.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	On campus Organized Research MTDC r	50.00	300,000.00	150,000.00
2.				
3.				
4.				
	Total Indirect Costs			150,000.00

Cognizant Federal Agency US Department of Health and Human Services, Arif Karim, 214-76

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	450,000.00

J. Fee

	Funds Requested ($)

K. * Budget Justification BudgetJustification1008827007.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 9297735540000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry *** Start Date:** 04/01/2016 *** End Date:** 03/31/2017 **Budget Period** 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Base Salary	EFFORT			0.00	0.00	0.00
2.	Dr.	Redacted by agreement				Co-Investigator					42,000.00	14,280.00	56,280.00
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 56,280.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
2	Post Doctoral Associates	EFFORT			80,000.00	27,200.00	107,200.00
	Graduate Students						
2	Undergraduate Students	12.00			20,800.00	208.00	21,008.00
	Secretarial/Clerical						
1	Microbiologist	EFFORT			0.00	0.00	0.00
5	**Total Number Other Personnel**					**Total Other Personnel**	128,208.00

Total Salary, Wages and Fringe Benefits (A+B) 184,488.00

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2016 * End Date: 03/31/2017 Budget Period 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	10,000.00
2.	Foreign Travel Costs	
	Total Travel Cost	10,000.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 3

Next Period

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2016 * End Date: 03/31/2017 Budget Period 3

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	89,200.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Per Diem	10,920.00
9. Blood Collection/Sample Processing	5,392.00
10.	
Total Other Direct Costs	105,512.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	300,000.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	On campus Organized Research MTDC r	50.00	300,000.00	150,000.00
2.				
3.				
4.				
	Total Indirect Costs			150,000.00

Cognizant Federal Agency US Department of Health and Human Services, Arif Karim, 214-76

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	450,000.00

J. Fee	Funds Requested ($)

K. * Budget Justification BudgetJustification1008827007.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 9297735540000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry *** Start Date:** 04/01/2017 *** End Date:** 03/31/2018 **Budget Period** 4

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Base Salary	EFFORT			0.00	0.00	0.00
2.	Dr.	Redacted by agreement				Co-Investigator					42,000.00	14,280.00	56,280.00
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 56,280.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
2	Post Doctoral Associates	EFFORT			80,000.00	27,200.00	107,200.00
	Graduate Students						
2	Undergraduate Students	12.00			20,800.00	208.00	21,008.00
	Secretarial/Clerical						
1	Microbiologist	EFFORT			0.00	0.00	0.00
5	**Total Number Other Personnel**					**Total Other Personnel**	128,208.00

Total Salary, Wages and Fringe Benefits (A+B) 184,488.00

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2017 * End Date: 03/31/2018 Budget Period 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	10,000.00
2.	Foreign Travel Costs	
	Total Travel Cost	10,000.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 4

Next Period

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2017 * End Date: 03/31/2018 Budget Period 4

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	89,200.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal Per Diem	10,920.00
9.	Blood Collection/Sample Processing	5,392.00
10.		
	Total Other Direct Costs	105,512.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	300,000.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	On campus Organized Research MTDC r	50.00	300,000.00	150,000.00
2.				
3.				
4.				
	Total Indirect Costs			150,000.00

Cognizant Federal Agency US Department of Health and Human Services, Arif Karim, 214-76

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	450,000.00

J. Fee

	Funds Requested ($)

K. * Budget Justification BudgetJustification1008827007.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 9297735540000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry *** Start Date:** 04/01/2018 *** End Date:** 03/31/2019 **Budget Period** 5

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Base Salary	EFFORT			0.00	0.00	0.00
2.	Dr.	Redacted by agreement				Co-Investigator					42,000.00	14,280.00	56,280.00
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 56,280.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
2	Post Doctoral Associates	EFFORT			80,000.00	27,200.00	107,200.00
	Graduate Students						
2	Undergraduate Students	12.00			20,800.00	208.00	21,008.00
	Secretarial/Clerical						
1	Microbiologist	EFFORT			0.00	0.00	0.00
5	**Total Number Other Personnel**					**Total Other Personnel**	128,208.00

Total Salary, Wages and Fringe Benefits (A+B) 184,488.00

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2018 * End Date: 03/31/2019 Budget Period 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
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6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	10,000.00
2.	Foreign Travel Costs	
	Total Travel Cost	10,000.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 9297735540000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Kansas State University

Delete Entry * Start Date: 04/01/2018 * End Date: 03/31/2019 Budget Period 5

F. Other Direct Costs		Funds Requested ($)
1.	Materials and Supplies	89,200.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal Per Diem	10,920.00
9.	Blood Collection/Sample Processing	5,392.00
10.		
	Total Other Direct Costs	105,512.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	300,000.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	On campus Organized Research MTDC r	50.00	300,000.00	150,000.00
2.				
3.				
4.				
	Total Indirect Costs			150,000.00

Cognizant Federal Agency US Department of Health and Human Services, Arif Karim, 214-76
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	450,000.00

J. Fee	Funds Requested ($)

K. * Budget Justification BudgetJustification1008827007.pdf Add Attachment Delete Attachment View Attachment
(Only attach one file.)

Obtained via FOIA by White Coat Waste Project

BUDGET JUSTIFICATION

Personnel

Roman R. Ganta, Ph.D., PI [EFFORT]. He will be responsible for overseeing all aspects of the project, including working with co-investigator and consultant. A microbiologist, two post-doctoral associates, and two student helpers will work under his direct supervision. No salary is requested.

[Redacted by agreement] Ph.D., Co-investigator [EFFORT]: She will oversee all aspects of the animal infection studies and will work closely with the PI, post-docs and other members of the research team. She will also be actively involved in weekly project meetings to discuss the research progress, data analysis and in preparing manuscripts.

[Redacted by agreement] Ph.D., Associate Professor [Redacted by agreement] has been an active collaborator of Dr. Ganta's for several years. Her input and contributions are significant in establishing the transposon mutagenesis in E. chaffeensis. She will continue to collaborate with the PI; on the current project, she will act as the consultant in providing input in experimental plan of developing a transposon mutant library for E. chaffeensis. No salary is requested. (Letter of support is attached.)

Post-doctoral associates ([EFFORT]): Two post-doctoral associates will be employed to receive research training and to perform experiments proposed in the project. We currently have two post-docs involved in the project [Redacted by agreement]. [Redacted] will focus mostly on the project goals for aim 1, while [Redacted by agreement] will be involved in aims 2 and 3. Both [Redacted by agreement] are major contributors in generating the preliminary data and are well-qualified to carry out the goals as proposed.

[Redacted by agreement], Microbiologist III ([EFFORT]): She will assist with culturing E. chaffeensis, transformation experiments, animal infection studies and with the routine laboratory maintenance. She will continue to serve as an important contributor for this project as she has a wealth of experience executing the project goals; she contributed significantly to the progress on the prior funded project. She will work with the post-docs, and other members of the research team. No salary requested.

Two student helpers (6 Cal. Months each): We will recruit two undergraduate students from Kansas State University to assist in animal handlings and sample collections, as well as to help in routine laboratory support. In the process, these students will also be provided hands-on research experience.

Supplies

Average monthly costs is $5,500 to obtain media reagents, sterile pipets and flasks, culture plates, pipets, gloves, antibody prep costs, PCR and centrifuge tubes, molecular biology reagents and kits, radioactive and other chemicals.

$23,200 per year is budgeted for the purchase of animals (28 dogs per year/$650 per dog) and related shipping fees ($5,000).

Travel

Travel funds are requested for the PI and appropriate personnel to attend a scientific conference(s) to present and obtain feedback on research progress.

Other Direct Costs

Animal per diem costs will be $10,920 per year ($6.5/day/dog x 60 days x 28 dogs).

$5,392 per year is budgeted for blood collection and sample processing fees.

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		281,400.00
Section B, Other Personnel		641,040.00
Total Number Other Personnel	25	
Total Salary, Wages and Fringe Benefits (A+B)		922,440.00
Section C, Equipment		
Section D, Travel		50,000.00
1. Domestic	50,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		527,560.00
1. Materials and Supplies	446,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	54,600.00	
9. Other 2	26,960.00	
10. Other 3		
Section G, Direct Costs (A thru F)		1,500,000.00
Section H, Indirect Costs		750,000.00
Section I, Total Direct and Indirect Costs (G + H)		2,250,000.00
Section J, Fee		

Obtained via FOIA by White Coat Waste Project

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Roman
Middle Name: Reddy
* Last Name: Ganta
Suffix:

2. Human Subjects

Clinical Trial? ☐ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Mr.
* First Name: Paul
Middle Name: R.
* Last Name: Lowe
Suffix:

* Phone Number: 785-532-6804
Fax Number: 785-532-5944
Email: plowe@ksu.edu

* Title: Asst Vice President/Research

* Street1: 2 Fairchild Hall
Street2:
* City: Manhattan
County/Parish: Riley
* State: KS: Kansas
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 66506-1103

Obtained via FOIA by White Coat Waste Project

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Obtained via FOIA by White Coat Waste Project

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☐ New ☒ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	Introduction1008827008.pdf	Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	SpecificAims1008826998.pdf	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	ResearchStrategy1008826999.p	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List	ProgressReportPublicationLi	Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	VertebrateAnimals1008826996	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	LetterOfSupport_Redacted by Agreement_100	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	ResourceSharingPlan10088269	Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

Obtained via FOIA by White Coat Waste Project

Page 183 of 430

Withheld pursuant to exemption

reviewers' comments

of the Freedom of Information and Privacy Act

Obtained via FOIA by White Coat Waste Project

SPECIFIC AIMS:

Illnesses caused by rickettsiales of the genera *Ehrlichia* and *Anaplasma* have been a growing health concern in recent years and are also the second leading cause of the tick-borne infections in the USA. They include human monocytic ehrlichiosis caused by *Ehrlichia chaffeensis* and human granulocytic ehrlichiosis and anaplasmosis resulting from *E. ewingii* and *Anaplasma phagocytophilum* infections, respectively. Recently, yet another new tick-borne pathogen, *E. muris* like agent, has been described as causing a disease in people. Despite the complex cellular environments of arthropods and vertebrates having sophisticated systems of defense, these rickettsiales evolved strategies to evade host clearance. For example, *E. chaffeensis* in macrophages and tick cells differ significantly in their morphology with distinct patterns of protein expression. The expression patterns include the differential expression from the multiple outer membrane proteins and effector proteins secreted via the pathogen's secretory pathways. For example, the p28-Omp multigene locus genes are differentially expressed; the p28-Omp gene 19 is highly expressed in macrophages, whereas in tick cells the expressed protein is predominantly the p28-Omp gene 14 product. Our studies also demonstrated that the differential expression in vertebrate and tick cells is conserved in other *Ehrlichia* species and that it contributes to the differential host response and delayed vertebrate host clearance. The central hypothesis of the funded NIH R01 project is that *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for pathogen's survival in vertebrate and tick cell environments. Specific aims of the funded project were to 1) establish *in vitro* transcription system for mapping *Ehrlichia* promoters, 2) map transcriptional machinery of two differentially expressed genes of the p28-Omp locus; genes 14 and 19, 3) evaluate the contributions of macrophage and tick cell environments for differential expression from genes 14 and 19, and 4) develop methods to evaluate knockout mutations to assess the biological relevance of host cell specific differential expression. We successfully accomplished the goals of aims 1 and 2. Goals of aim 3 are a work in progress and although much has been accomplished, some of the novel directions we took during the last funding period required additional expansion of experiments in this aim; it will be part of the renewal application (aim 1). Aim 4 was the most innovative and the most challenging because to date there are no methods described in the literature which aid in introducing foreign DNA and creating mutations in *Ehrlichia* species. We had to do a lot of ground work in achieving this goal, and indeed we have succeeded in accomplishing this challenging task. We now have protocols in place for creating both targeted and random mutations in *E. chaffeensis*. We have convincing evidence showing that stable gene inactivation mutations can be developed in *E. chaffeensis*; we developed mutations in several genomic locations of the organism, including within the coding regions of some genes. We also have presented the first evidence of identifying three genes essential for the pathogen's growth in vertebrate host. We accomplished this by utilizing an *in vivo* screening method which we established. These significant novel data, generated with support for the previous funding cycle, form the strong foundation for the goals we set for this competing renewal application having the following highly focused specific aims. The aims remain focused to continue testing our central hypothesis (outlined above).

Specific Aims:

1) Characterize E. chaffeensis RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression

2) Evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for E. chaffeensis in vivo growth

3) Perform mutational analysis and in vivo screening to identify additional genes essential for the E. chaffeensis pathogenesis in vertebrate and tick hosts

We established all of the needed methods to accomplish the goals of the proposed project. We are the first and the only group in developing mutational methods. Likewise, we are the first and the only group in undertaking studies focused on defining the regulation of gene expression by *Ehrlichia*; all of the needed molecular tools for mapping the gene expression have been developed by us. We also carefully reevaluated and standardized additional research tools needed for advancing our understanding of the *Ehrlichia* pathogenesis; they include the establishment of infection studies in hosts involved in the transmission cycle of the pathogen, the white-tailed deer and its tick vector, *A. americanum.* Novel data in this revised submission include validating the use of a dog infection model. The infection studies in deer, dog and tick are critical in asking key questions about the pathogenesis of the organism. This collaborative study with investigators having expertise in *Ehrlichia* molecular biology and animal studies brings in the needed strength to accomplish the goals set for this innovative research having important implications in moving the *Ehrlichia* field significantly forward.

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SIGNIFICANCE: The family *Anaplasmataceae* contains several obligate, intracellular, Gram-negative bacteria which include species of the genera *Ehrlichia* and *Anaplasma* [1]. A steady increase of potentially fatal human diseases caused by the species of the genera *Ehrlichia* and *Anaplasma* that infect phagocytic cells has been reported during the last 25 years [2-5]. These include three tick-transmitted emerging diseases of humans caused by *Ehrlichia* species: human monocytic ehrlichiosis (HME) caused by *E. chaffeensis* [6,7], human ewingii ehrlichiosis caused by *E. ewingii* [8], and a more recently identified infections with *E. muris* like agent [5]. Further, infections in people are also caused by *Anaplasma phagocytophilum* resulting in the disease, human granulocytic anaplasmosis (HGA) [9,10]. Tick-transmitted pathogens must have strategies to persist in both tick and vertebrate hosts in order to successfully complete their infectious cycle. Unlike insect vectors, the probability of transmitting a pathogen from a tick to a vertebrate host and similarly vertebrate host to tick would be significantly low. Thus, it is necessary for tick-transmitted pathogens to have evolved strategies to establish persistent infections in both vertebrate and tick hosts. Indeed, it is widely documented that tick-transmitted rickettsiales of the genera *Ehrlichia* and *Anaplasma*, including *E. chaffeensis,* establish persistent infections [11-20].

An infectious disease is the result of a pathogen's ability to overcome host clearance. It is important to determine how the pathogens overcome the host challenge in support of completing their transmission cycle. Much of the recent research suggests that the *Anaplasmataceae* pathogens express several proteins on their cell surface, into the host phagosome or into the cytoplasm in support of their survival and growth in the host cell environments [21-24]. Intriguing data are presented demonstrating that some of the secreted proteins are transported to the host cell nucleus or mitochondria [24-27]. We and others presented evidence that *Ehrlichia* species alter the expression of many proteins in a host cell-specific manner when replicating in vertebrate and invertebrate host cells [28-30]. Our previous studies demonstrate that the major expression from the p28-Omp multi-gene locus from *E. chaffeensis* is limited only to a subset of genes and the expressed proteins from this locus are unique to vertebrate and tick cells [31]. The p28-Omp antigens may be essential components for the pathogen's survival in adapting to multiple hosts and to establish persistent infection. The adoptive transfer of antibodies against the p28-Omp antigens of *E. chaffeensis* is protective against fatal infections assessed in SCID mouse model [32]. Preliminary evidence suggests that these outer membrane expressed proteins function as porins [33,34]. The clearance of *E. chaffeensis* by a host is delayed when it is grown in tick cells as compared with those grown in vertebrate host cells [35]. Furthermore, the host response differed considerably from *E. chaffeensis* originating from tick and vertebrate host environments [35]. Our research has the primary focus of gaining fundamental knowledge important for the *in vivo* growth of *E. chaffeensis*, assessing the feasibility of such knowledge in devising methods of controlling the infection acquisition, and understanding the regulation of the pathogen gene expression in vertebrate and invertebrate host environments.

INNOVATION: Much of the fundamental knowledge about how the host-specific gene expression is accomplished and how it contributes to the pathogen's persistence can be understood by studying the regulation of gene expression. Likewise, defining the importance of one or more pathogen proteins to the growth and pathogenesis of the organisms requires methods to be able to knockout or knockdown the protein expression. The goals we set and accomplished during the previous R01 funding were very challenging and innovative. For example, we established the *in vitro* transcription system, as it offers an innovative means to understand how *E. chaffeensis* and other related pathogens alter gene expression by sensing the host cell environments. In the absence of an established genetic manipulation system, defining the gene function *in vitro* opens a novel way for understanding the importance of the pathogen's differential gene expression. Although similar studies have been actively pursued for another obligate intracellular pathogen for which mutational methods are also limited, *Chlamydia trachomatis* [36,37], we are the first and the only group to introduce such methods to rickettsiales. We also had another challenging and highly innovative goal in our previous application, that is, to establish a genetic manipulation system. We did succeed in establishing both targeted and random mutagenesis methods. We also standardized two infection animal models assessing pools of mutants (deer and dog) to identify genes essential for the *E. chaffeensis* growth *in vivo*. Our research during the last project period, therefore, was focused on defining the promoter regions of several *E. chaffeensis* genes [38,39], purifying and characterizing the native RNA polymerase (RNAP) holoenzyme [38,39] and preparing recombinant sigma factors (σ32 and σ70) and using them to reconstitute functional RNAP holoenzyme and establishing the *in vitro* transcription methods to map the pathogen gene promoters [38,39], and developing methods to map the functional domains of *E. chaffeensis* promoters in various modified strains of *E. coli* [38,39]. Further, we studied the molecular (proteomic and transcriptomic) and morphological changes in the organism [40]. We also reported recently the characterization of a molecular chaperone, ClpB, in an effort to understand how *E. chaffeensis* survives the host-induced stress [41]. Further, we have worked in developing methods for creating both targeted and random mutations in *E. chaffeensis* [42]. We reported the establishment of stable gene inactivation mutations in *E. chaffeensis.* We created mutations in several genomic locations, including in genes differentially expressed. Furthermore, we developed an *in vivo* screening method by performing infections in white-tailed deer, dog and in *Amblyomma americanum* tick to present the first evidence of identifying genes essential for the pathogen's growth in its hosts [42]. Deer, dog and tick infection studies, although challenging, are ideal for mapping essential *E. chaffeensis* genes as they

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are the recognized as acquiring infections with the pathogen. The dog is an ideal model for these studies because, like humans, it is considered as an accidental host for *E. chaffeensis* [43,44]. These significant novel data form the strong foundation for the innovative goals we set for this competing renewal application.

PROGRESS REPORT FOR THE PREVIOUS FUNDING CYCLE: We received funding from 11/29/2007 to 11/28/2012 (5 years project plus one year no cost extension). The project included four specific aims. Progress on all four aims is briefly described below.

Specific Aims 1 and 2: 1) Establish *in vitro* transcription system for mapping *Ehrlichia* promoters and 2) map transcriptional machinery in differentially expressed genes of the p28-Omp locus. The focus of these two aims was to establish *in vitro* transcription assays using recombinant or/and native *E. chaffeensis* RNA Polymerase (RNAP) complex, prepare various deletion constructs of two differentially expressed gene promoters and assess their promoter activities. We accomplished these goals and the research results were published [38,39]. Pprogress from these two aims and additional directions we took are described below.

In vitro transcription: The *in vitro* transcription assay was developed using the promoters of *E. chaffeensis* p28-Omp 14 and 19 genes. We published these data describing the initial assay development and its application in mapping the promoter segments [38]. The assay was further improved to reduce the non-specific *in vitro* transcribed products by utilizing a G-less cassette construct, similar to the one described for *in vitro* transcription studies on *C. trachomatis* [45]. Moreover, the assay was utilized in mapping three more *Ehrlichia* gene promoters (*dnaK, hup* and *GroE*) (manuscript in review; Unpublished).

E. chaffeensis RNA Polymerase (RNAP) purification: *E. chaffeensis* RNAP holoenzyme was purified by the heparin agarose purification method. The enzyme was characterized to identify optimal conditions of the divalent and monovalent cation specificity. The bacterial origin of the enzyme was confirmed by performing immunoblotting analysis. Further, the enzyme specificity was confirmed by its rifampin and *E. coli* σ70 antibody-mediated inhibition assays. The data are described in [38].

Recombinant σ70 (RpoD gene product) subunit preparation and its use in *in vitro* transcription: As a backup plan to native *E. chaffeensis* RNAP purification, we constructed an expression plasmid containing the *E. chaffeensis* RpoD gene (encoding σ70) by utilizing the pET32 expression system (Novagen, Billerica, MA). The recombinant protein was purified and used to form holoenzyme by combining with *E. coli* core enzyme. The holoenzyme, but not the core enzyme alone, is active in driving expression from the *E. chaffeensis* gene promoters. The recombinant σ70-properties were assessed and are similar to purified native enzyme [38].

Considering the technical challenges associated with gene regulation studies utilizing the *in vivo* system for an obligate intraphagosomal pathogen, an *in vitro* transcription system can serve as an important molecular tool to map differences in *E. chaffeensis* gene expression. This is particularly important since established genetic manipulation methods were also not available for mapping *E. chaffeensis* genes. Extensive studies have been reported using similar *in vitro* transcription system to map regulation of gene expression for another intraphagosomal pathogen, *C. trachomatis,* for which genetic manipulation system is also not well developed [46,47]. In our studies, we mapped differences in the promoter regions of two differentially expressed genes of the p28-Omp multigene locus, genes 14 and 19. The promoters were evaluated to map differences in gene expression and to locate RNAP binding motifs. RNA and promoter deletion analysis aided in identifying transcription differences and DNA sequences that influenced promoter activity. Experimental evidence was presented demonstrating that the regulation of gene expression is achieved by changes in RNAP activity, possibly due to altered curvature of sequences upstream to the RNAP binding site or via interaction of transcription regulators which may be differentially expressed in macrophages and tick cells [39].

Additional progress in Specific Aim 2 (*Note: the data described here were compiled as a manuscript and submitted to the peer-review journal* Unpublished). *In vitro* transcription assays were used in mapping various segments of the differentially expressed gene promoters. We made significant progress in understanding of how differential expression is accomplished and which domains of the promoters contribute to the RNAP activity. These studies were carried out with two genes influenced by the host cell environments (tick and macrophage). These promoters were recognized by the sigma factor, σ70. We also expanded studies to genes actively transcribed with RNAP holoenzyme containing σ32 (RpoH gene product). Although the research involving the σ32 was not a proposed goal for the project, we reasoned that it is important to investigate because it is the only alternate sigma factor in the *E. chaffeensis* genome and likely an important contributor for differential gene expression. The data defining the σ32 promoters and its comparison to the σ70 promoters are summarized below. (Additional data that were not described here were included in the manuscript submitted to Unpublished

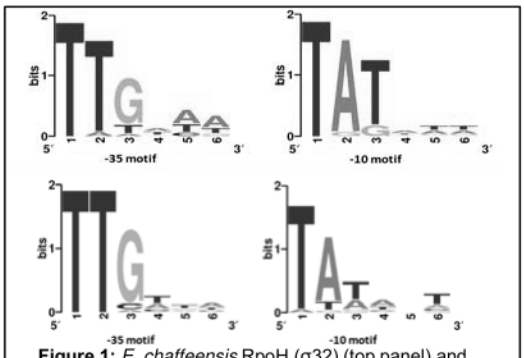

Figure 1: *E. chaffeensis* RpoH (σ32) (top panel) and RpoD (σ70) (bottom panel) consensus RNAP binding motifs, -10 and -35.

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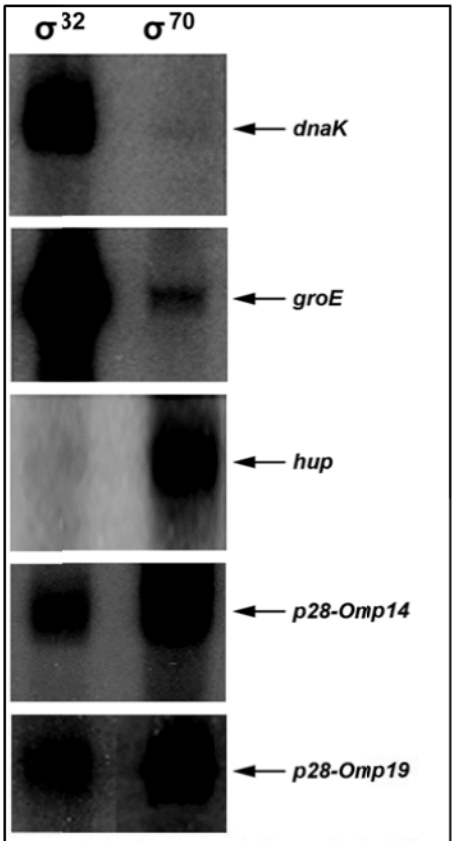

Figure 2: In vitro transcription analysis of the promoter segments of dnaK, groE, hup, p28-Omp14 and 19 genes. The gel images show transcripts produced by E. chaffeensis recombinant sigma 32 and sigma 70 from the wild-type promoters.

Identification of *E. chaffeensis* σ32 and σ70 promoters: *E. chaffeensis* genome contains genes only for two sigma factors [48]; the primary housekeeping σ70 and the alternate stress response factor, σ32. We evaluated the transcriptional machinery of several pathogen genes likely be transcribed by its σ32. Prediction analysis was performed to identify the transcription start sites (TSS) and RNAP binding motifs (-35 and -10 domains) of *E. chaffeensis* genes likely to be recognized by the RpoD (28 genes) and RpoH (19 genes) by utilizing the NPP2.2 [49] (http://www.fruitfly.org/seq_tools/promoter.html) and WCONSENSUS programs (http://stormo.wustl.edu/consensus/html/Html/main.html). Consensus -35 and -10 domains of *E. chaffeensis* σ32 are TTGAAA and TATATT, respectively, and they are very similar to σ70 consensus sequences (TTGTTA and TATANT, respectively) (**Figure 1**) [39]. We previously mapped -35 and -10 domains of two *E. chaffeensis* σ70 promoters and demonstrated that the deletion of -35, but not -10 motif, results in significant decline in the transcription [39]. The consensus -35 domain of *E. chaffeensis* σ32 genes is identical to the *E. coli* consensus sequence, whereas its consensus -10 domain differed significantly to *E. coli* sequence (CCCCATNT) [50]. TSS for 10 putative promoters were mapped by primer extension or 5' RACE analysis and they were located in close proximity of the putative binding domains, -35 and -10.

***In vitro* transcription for *dnaK*, *groE* and *hup* promoters by recombinant *E. chaffeensis* RpoH and RpoD**: High degree of conservation and shared similarities in the -35 and -10 motifs of σ32 and σ70 promoters in *E. chaffeensis* suggest that the gene promoters are likely to be recognized by both the sigma factors in driving transcription. To test this hypothesis, we expressed and purified the *E. chaffeensis* σ70 [51] and σ32 (new data) and performed *in vitro* transcription assays. The promoter sequences of *dnaK, groE* and *hup* (putative σ32 genes) were cloned into the G-less cassette plasmid to serve as the transcription templates. We previously created constructs for two σ70 promoters, *p28-Omp 14* and *p28-Omp 19* [39]. *In vitro* transcription from the holoenzyme with the recombinant σ32 or σ70 resulted in transcription from all five promoters (**Figure 2).** Importantly, all five promoters were recognized by both transcription factors, although transcripts are higher with the σ32 for the *dnaK* and *groE* promoters than those made with the σ70, whereas the opposite is true for the promoters of *hup*, *p28-Omp 14* and *p28-Omp 19* (**Figure 2**). The data demonstrate that σ32 and σ70 in *E. chaffeensis* are not exclusive in their promoter recognition, although affinity differences may exist.

To further define the *E. chaffeensis* σ32 promoters, we developed an *in vivo* assay in an *E. coli* strain (CAG57101) where its chromosomal σ32 gene is inactivated [52] and in its place we expressed *E. chaffeensis* σ32 by transforming with a plasmid which is under the control of IPTG inducible p-Lac promoter. The *E. coli* strain was also transformed with recombinant plasmid containing *E. chaffeensis* promoter segments of *dnaK*, *groE* or *hup* cloned upstream to the promoterless β-galactosidase gene or with promoterless plasmid. We reasoned that the *E. coli* RNAP core enzyme forms a functional holoenzyme with *E. chaffeensis* RpoH (similar to *in vitro* experiments) after its induced expression with IPTG and drives transcription in *E. coli*. Indeed, induction of the *E. chaffeensis* σ32 expression resulted in the increase of specific activity of all three promoters in *E. coli*, as assessed for the β-galactosidase specific activity (**Figure 3**). This

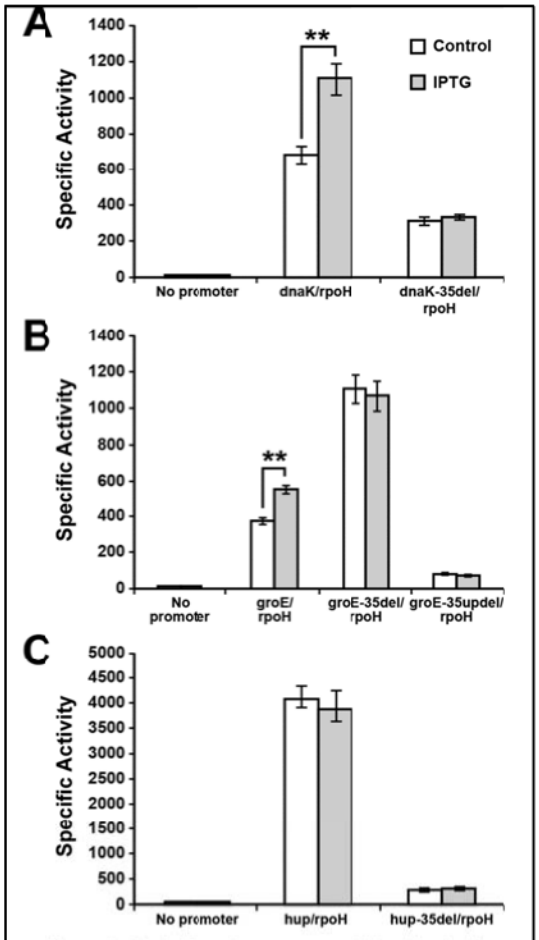

Figure 3. *E. chaffeensis* promoter activities (A, *dnaK*; B, *groE*; and C, *hup*) assessed in *E. coli* by measuring the β-galactosidase expression. The β-galactosidase expression driven by *E. chaffeensis* promoters from the wild type promoters (*dnaK, groE* or *hup*), promoters containing -35 motif deletion (*dnaK-35del, groE-35del* or *hup-35del*) or *groE* promoter having complete deletion from -35 to the entire upstream sequence (*groE-35updel*) were measured in the CAG57101strain of *E. coli* before or after the induced expression of *E. chaffeensis rpoH*. The CAG57101 strain contained either promoterless pQF50K (control) or one of the pQF50K-Ech_promoter plasmids together with the pSAKT32-Ech_rpoH plasmid. (Data from 3 experiments with standard deviation were presented.) Significant changes were identified where the *P* values were ≤0.01.

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model is very similar to several studies reported in the literature [23,52,53]. The maximum enzyme activity was observed for the *dnaK* promoter (138%), followed by the *groE* promoter (60%), i.e., above the background level observed in the absence of RpoH. The specific activity for the *hup* promoter is only minimally enhanced with RpoH (16%). In controls where *E. chaffeensis* RpoH negative plasmid was transformed, the specific activity of the *dnaK* and *groE* promoters was about 400 units, whereas for the *hup* promoter, it had about 8 times higher activity (3,400 units). The *hup* was found to be predominantly σ70-specific promoter (as per *in vitro* transcription data), thus we reasoned that the high *hup* promoter activity in *E. coli* is due to its recognition by the *E. coli* σ70. The transcription increase from *dnaK* and *groE* promoters in the σ32 mutant *E. coli* strain following inducing the *E. chaffeensis* σ32 is consistent with the *in vitro* transcription data, thus validating that the *dnaK* and *groE* promoters have higher affinity for the σ32, whereas the *hup* promoter has a greater affinity for the σ70. In the absence of *E. chaffeensis* σ32, the pathogen promoter constructs also exhibited some β-galactosidase activity, suggesting that they are also recognized by the *E. coli* σ70. This is also consistent with the prior study demonstrating that *E. coli* RNAP does recognize *E. chaffeensis* promoters [39].

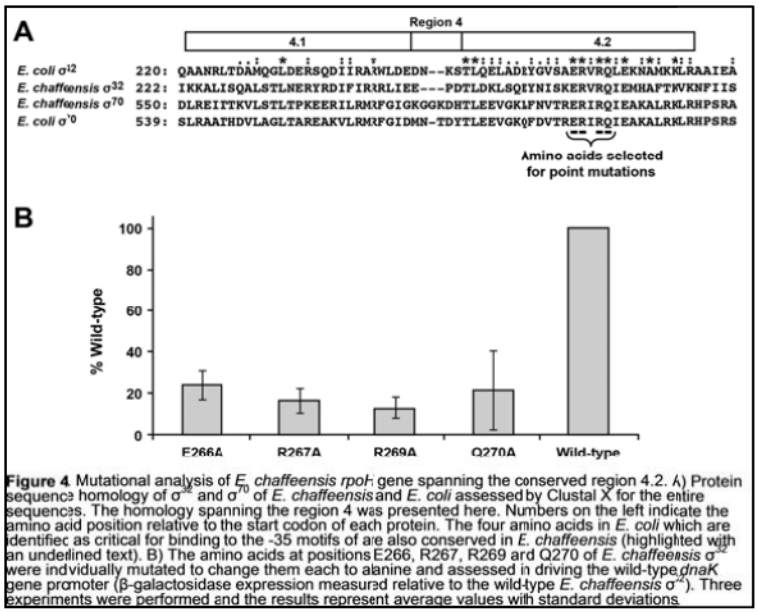

Figure 4. Mutational analysis of *E. chaffeensis rpoH* gene spanning the conserved region 4.2. A) Protein sequence homology of σ^{32} and σ^{70} of *E. chaffeensis* and *E. coli* assessed by Clustal X for the entire sequences. The homology spanning the region 4 was presented here. Numbers on the left indicate the amino acid position relative to the start codon of each protein. The four amino acids in *E. coli* which are identified as critical for binding to the -35 motifs of are also conserved in *E. chaffeensis* (highlighted with an underlined text). B) The amino acids at positions E266, R267, R269 and Q270 of *E. chaffeensis* σ^{32} were individually mutated to change them each to alanine and assessed in driving the wild-type *dnaK* gene promoter (β-galactosidase expression measured relative to the wild-type *E. chaffeensis* σ^{32}). Three experiments were performed and the results represent average values with standard deviations.

Earlier, we reported that the -35 motifs are critical for the σ70 binding [39]. We reasoned that the -35 motif for σ32 genes is similarly important; we created constructs lacking this entire motif from all three promoter segments (*dnaK*, *groE* and *hup)*. The -35 deletions from *dnaK* and *hup* resulted in the significantly reduced β-galactosidase activity (to the background levels as observed in the absence of a promoter), whereas the specific activity of *groE* promoter was significantly increased and nearly doubled as compared to its wild-type promoter. As there is second consensus -35 motif (TTGATA) directly upstream to the deleted -35 motif (not shown), we reasoned that this motif was shifted to the deleted -35 position and served as the new motif to functionally complement the σ32 function. To verify this, we deleted the entire *groE* promoter segment upstream to the first -35 motif and assessed for the promoter activity. This deletion caused the loss of specific activity to background level (**Figure 3**). Together, these data validate our working hypothesis that there is a significant overlap in the promoter recognition by σ32 and σ70 factors, although some specificity differences do exist.

Substitutions in region 4.2 of *E. chaffeensis* RpoH: Sequence alignment of *E. chaffeensis* σ32 and σ70 with those of *E. coli* revealed extensive amino acid conservation. Previous research in *E. coli* suggests that four amino acids in region 4.2 of the σ32 (E265, R266, R268 and Q269) are essential for binding to the -35 motif of the promoter sequences [54] and these amino acids are also conserved in the *E. chaffeensis* (**Figure 4**). In particular, these four amino acids are considered essential for the -35 recognition in *E. coli* [54]. To assess if these amino acids are similarly critical, mutational analysis were performed in *E. chaffeensis* RpoH gene. Specifically, substitutions were made in the sequences spanning the conserved amino acids in *E. chaffeensis* RpoH gene and were assessed for their impact on the promoter activity of *dnak.* Substitutions at all four amino acids caused a significant decline in the transcription (80% decrease) **(Figure 4)**.

Previous studies indicated that the -35 motif of the σ32-dependent genes show the sequence conservation of TTG residues in all proteobacteria, regardless of subdivisions [55-61]. The -35 motif in *E. chaffeensis* σ32 dependent promoters also contain the same conserved TTG motif. Moreover, it is similarly conserved in *E. chaffeensis* σ70 promoters which further support the shared recognition of genes by σ32 and σ70.

Summary: To understand gene regulation in *E. chaffeensis,* we conducted studies evaluating the transcriptional machinery of several pathogen genes. We mapped the promoters of *GroE, dnaK, hup, p28-Omp14* and *p28-Omp19* genes. We showed that the RNAP binding motifs of the pathogen gene promoters share greater homology for σ32 and σ70, the only two transcription regulators of *E. chaffeensis,* and that the pathogen gene expression is accomplished by either one of the regulators. The data suggest that *E. chaffeensis* gene regulation is evolved distinctly to support the pathogen's growth, possibly to promote its intra-phagosomal growth. Considering the limited availability of genetic tools, this study offers an innovative approach in defining gene regulation in *E. chaffeensis* and in other related pathogens. The progress outlined is important also for advancing the understanding of the stress responses by *E. chaffeensis*. We also investigated a stress response regulatory protein of *E. chaffeensis*, ClpB, and the work was reported recently in [41]. These data were not included here due to space constraints, but the manuscript is available online. In summary, the progress

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described in understanding the gene regulation further warrants the need for continued research in this virtually unexplored territory.

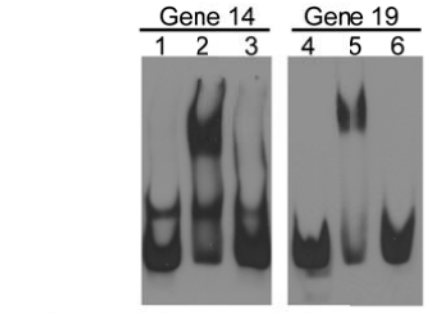

Figure 5: Gel mobility shift assays were performed on biotin-tagged promoter segments of p28-Omp 14 and 19 genes (Ech_1136 and Ech_1143, respectively) with the recombinant protein of Ech_0795 (ApxR homolog) and detected with avidin conjugate. Competitor is 100 fold molar excess of the probe with no biotin tag.

Specific Aim 3: Evaluate the contributions of macrophage and tick cell environments for differential expression from genes 14 and 19 of the p28-Omp locus: In support of accomplishing the goals of this aim, we standardized the protocols for mobility shift assays; *E. chaffeensis* protein preparations were made from the organisms cultured and purified from the macrophage and tick cell lines. We observed shift in the promoter regions when *E. chaffeensis* protein preparations were added. We also mapped various segments of the p28-Omp 14 and 19 promoters where the proteins bound [39]. This is the first step in identifying the regulators associated in modifying host-specific gene expression. Since our previous submission, we made substantial progress in this aim; we recombinantly expressed and purified 5 of 8 predicted DNA binding proteins of *E. chaffeensis* (products of genes ECH_1118, ECH_0795, ECH_0804, ECH_1012, and ECH_0163 were expressed) and utilized to assess if these proteins contribute to differential gene expression. We identified one regulatory protein, product of Ech_0795 gene, as interacting specifically with the promoter segments of two differentially expressed p28 Omp genes (Ech_1136 and Ech_1143) as assessed by binding of the proteins to promoter segments examined by gel mobility shift assay (**Figure 5**). {The specific interaction was verified by its not binding to three other gene promoters (not shown) and the abolition of binding by cold competitors.} Interestingly, it is a homolog of *ApxR* of *A. phagocytophilum* that is shown to control gene expression of the pathogen's outer membrane expressed p44 protein [62]. In particular, p44 expression is high in the organisms grown in promyelocytic human leukemia cell line (HL-60) and in spleen of infected mice, whereas its expression is significantly low in ISE6 tick cells and in infected tick salivary glands [62]. Furthermore, *ApxR* expression paralleled p44 expression patterns; its expression is also significantly high in *A. phagocytophilum*-infected HL-60 cells than in infected ISE6 tick cells. These data suggest that, the ApxR homolog in *E. chaffeensis* may represent a likely regulatory protein impacting differential gene expression of p28-Omp proteins. Together, we believe that we made substantial progress in identifying a regulatory protein as interacting with the differentially expressed gene promoters of *E. chaffeensis*. More work needs to be done in defining how host cell environments alter gene expression; this research will continue as part of Aim 1 of the competing renewal application.

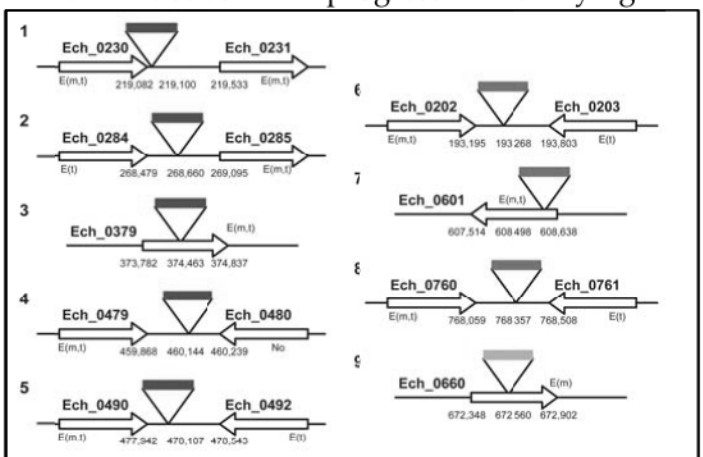

Figure 6: A cartoon illustration of the E. chaffeensis genomic locations mapped for the transposon mutants (three independent experments were performed; one mCherry and two GFPuv containing Himar1 transposon plasmids). Genomic integrations assessed by PCR and sequence analysis. Gene expression data were also included (E, expressed genel m, in mancrophage cultures; t, in tick cel culture; No, gene not expressed)

Specific Aim 4: Evaluation of knockout mutations to assess the biological relevance of host cell specific expression. Research to elucidate gene function in rickettsial pathogens is limited by the lack of genetic manipulation methods. The goal of this aim was to develop knockout systems useful in testing the hypothesis that unique host cell-specific gene expression is essential for the pathogen's survival in vertebrate and tick cell environments. This was the most challenging goal; our methodical approach aided in making significant progress. We surveyed the global gene expression of *E. chaffeensis* originating from macrophage and tick cells by performing proteome and microarray analysis. Results from proteome analysis were published [28,29] and the microarray data are compiled as the manuscript and it will soon be communicated [Unpublished unpublished). (Due to space constraints, data were not shown.) The functional genomics data were valuable in executing mutational experiments.

Targeted mutagenesis: A major hurdle in advancing research in *Ehrlichia* species has been the lack of genetic tools. We took a methodical approach in creating both targeted and random mutations in *E. chaffeensis*; 1) we evaluated various experimental conditions to identify protocols that work the best in

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transforming the organism; 2) we assessed various antibiotics for identifying drugs that are useful in screening the mutants; 3) we tested the feasibility of creating targeted mutations by homologous recombination; we expanded the targeted mutagenesis to six genomic locations using the modified mobile group II intron method, as recent studies suggest that it is an efficient method in creating mutations in bacteria [63-66]. The method aided in the consistent identification of mutations in three targets, including one located within a differentially expressed gene (Ech_1143) encoding an outer membrane protein, p28-Omp 19. The mutants, however, survived in culture only for a short period of 8 days. We reasoned that genomic sites where mutants could not be detected represent essential genomic regions required for *E. chaffeensis* growth *in vitro*. Two interesting results were; 1) we discovered that targeted mutation in the predominantly macrophage-specific outer membrane protein gene of p28-Omp 19, a paralog of the p28-Omp 14 gene (Ech_1136), had no impact on the growth of the organism in macrophage cultures. 2) In contrast, mutants could not be detected when targeted to the primarily the tick cell-specific expressed p28-Omp 14 gene. We reasoned that in the absence of p28-Omp 19, its function may be complemented by one of the remaining 21 paralogs, as all of them are expressed at low levels in macrophage infected organisms [29,34,67], whereas the p28-Omp 14, the major expressed protein in tick cells which also is expressed in macrophages, may represent an essential protein for the bacterium's growth [35].

Transposon mutagenesis: We evaluated transposon-based random mutagenesis and demonstrated that stable *E. chaffeensis* mutants could be generated by this method. Mutations at nine genomic locations were identified (**Figure 6**) [42]. We presented evidence that the transposon mutants are a heterogeneous mix of clones. We mapped all 9 mutation sites in the *E. chaffeensis* genome; five were located in non-coding regions and had no impact on the expression of genes surrounding the mutation sites. Mutations in four locations caused inactivation of gene activity (Ech_0230, Ech_0379, Ech_0601 and Ech_0660). Growth of three disruption mutations in differentially expressed genes (Ech_0230, Ech_0379, Ech_0660) caused growth attenuation when assessed in white-tailed deer, the natural reservoir host for the pathogen [42]. The nine genomic locations are the first to be identified as non-essential for *E. chaffeensis* growth *in vitro* and the data are reported in [42]. Altered growth rates in some mutants can also result in the lack of *in vivo* detection; our infection studies with clonally purified organisms verified that the primary reason for attenuation in not the result of altered mutant growth rates (new data described below).

Animal infection studies to identify genes required for *in vivo* growth: We performed infection studies in deer (three animals) with pools of mutants and their growth was assessed over a period of 60 days by sampling blood at 3 to 4 day intervals. The analysis included the culture recovery and/or by nested PCR targeting to each mutation insertion. The only mutants consistently detected were those that did not impact the gene expression and also the mutant having gene inactivation in Ech_0601. Three mutations impacting the loss of gene expression (Ech_0230, Ech_0379 and Ech_0660) were undetectable in deer. Similarly, these three mutants were negative in ticks acquisition-fed on the infected deer. Failure of tick acquisition of gene disruption mutants is likely due to their lack of productive infection. These data suggest that the elimination of some mutants *in vivo* is the result of their growth attenuation. One possibility, however, does exist that the altered growth kinetics of some mutants may contribute to non-detectable growth of organisms in deer. We investigated this hypothesis by repeating the infection experiments in deer and dog with two clonally purified mutant organisms; one that grew normally in deer (Ech_0284) and a second mutant that did not grow (Ech_0660). We also performed infection with another clonally purified mutant, Ech_0379, in dogs. The deer infection data were reported in [42] and the dog infection data were summarized below.

Functional significance of genes important for *in vivo* growth: The gene Ech_0230 encodes for a putative membrane protein and Ech_0379 and Ech_0660 are identified as genes encoding hypothetical proteins [48] (GenBank # CP000236.1). The gene product for the Ech_0660 contains a putative conserved domain having significant homology to proteins of phage capsid assembly (BLAST analysis data), suggesting that it also is a membrane expressed protein. Protein database search on the translated coding sequence of Ech_0379 revealed a significant homology with membrane-bound, monovalent cation (Na^+)/H^+ antiporter proteins of *Corynebacterium* species. Further, our secondary structure prediction analysis of Ech_0379 protein [68] identified the C-terminus sequence containing a transmembrane domain. Similarly, Ech_0230 had a predicted secondary structure having 10 transmembrane domains [42]. Homologs of Ech_0379 are also present in other *Ehrlichia* species and are classified as integral membrane proteins [42]. *E. chaffeensis* resides in a phagosome environment and most likely depends heavily on antiporters for its pH balance in a vertebrate host. Together, the predicted products of all three genes (Ech_0230, Ech_0379 and Ech_0660) are associated with membrane structures.

Summary: We developed both targeted and random mutational methods at several regions of *E. chaffeensis* genome. The mutational analysis data were published recently [42]. It is the first report demonstrating the mutations in *Ehrlichia* species and directly linking specific regions of the genome to the pathogen growth *in*

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vivo. Molecular tools developed in our studies are significant in advancing the detailed understanding of the role of specific genes for the dual host life cycle of this pathogen and the primary focus of Aims 2 and 3 of this application. The methods established in our previous funding cycle are critical for screening the *E. chaffeensis* genome in identifying essential genes in understanding the pathogenesis.

NEW PRELIMINARY DATA SINCE OUR LAST SUBMISSION: **Dog as an infection model:** As deer is a natural host for *E. chaffeensis*, a concern was raised about its use as an animal model for studies focused on understanding the pathogenesis relating to infections in people. The pathogen may be better adapted to a natural host in support of its successful propagation in deer in completing its lifecycle, as opposed to humans who are accidental hosts. Two alternate possible animal models for screening mutants are mouse and dog. We have considerable expertise in utilizing the murine host for *E. chaffeensis* infection studies [35,69,70]. In particular, we utilized the murine host in studies focused on understanding the immune responses. While many immunological reagents are available and indeed it is a great model for understanding which vertebrate host immune components are important for generating protective response, one major challenge with the murine host is that *E. chaffeensis* infections do not persist in this host (infection clears in less than 14 days-time) [35] and moreover it is not known to acquire the pathogen naturally. The lack of persistent infections in mice would be a particular a challenge for our proposed studies in investigating the goals of Aims 2 and 3. Alternatively, dog is known to acquire *E. chaffeensis* infections naturally by a tick bite and is known to exhibit clinical signs [43,44]. Experimental infection studies by Zhang et al [20] demonstrated that the beagle dog breed is well-suited for infection experiments where the animals do not exhibit severe clinical signs and the pathogen persists for up to four months or longer. We conducted a preliminary study to reevaluate if infections with *E. chaffeensis* persist in the dog model. With IACUC approval, we conducted a preliminary study. Specific pathogen free beagles were purchased from a commercial breeder (Covance Research Products, Denver, PA) and housed at Kansas State University climate controlled indoor animal facility. Specifically, two animals each were infected intravenously with either wild-type *E. chaffeensis* organisms or with a pool of 9 transposon mutants or with clonal populations of Ech_0284 (one dog), Ech_0379 or Ech_0660 mutants. Dogs were monitored for clinical signs and blood was sampled twice a week for up to 44 days for hematological changes and for the presence organisms by PCR targeting *E. chaffeensis*-specific 16S rRNA gene (wild-type infected samples) or for the presence of specific mutants by performing mutant-specific PCRs (in mutant infected dog samples). Blood samples were also assessed by *in vitro* culture (Table 1). *E. chaffeensis* positives were identified at several post-infection days starting from day 2. Wild-type and 9 mutant pool infected animals were sacrificed at day 44 and various tissue samples were also assessed. The pathogen was found in liver, lymph node and spleen (PCR analysis). Interestingly, the mutants recovery data from dogs are consistent with those observed in deer (Table 1); the data validate that the dog is an ideal animal model for infection studies with *E. chaffeensis*, as it is an accidental host like humans. It also supported the persistent growth of the organism. Finally, we observed the same three mutants found to be attenuated in deer were also attenuated when assessed in dogs. Furthermore, *E. chaffeensis* was not detectable in deer or dog when infections were repeated with clonally purified organisms having a gene function disruption mutation at Ech_0660. Similarly, mutation in Ech_0379 resulted in attenuated growth in the canine host (PCR positives were detection only on day 2 post-infections for both Ech_0379 and Ech_0660, but are negative for all other days). ***These data clearly support the hypothesis that the gene disruption mutations in putative membrane protein genes (Ech_0230, Ech0379 and Ech_0660) are truly attenuated and not the result of altered growth kinetics.*** (Note: Ech_0660 clonal infection in deer was reported in [42], while the dog infection study is work-in-progress).

Table 1: Mutants persisted/cleared similarly in both natural (white-tailed deer) and accidental (dog) hosts

Deer infection data*: Mutants assessed in blood of animals infected as pool of mutants or as clonal population

Mutant #	Ech_0202	Ech_0230	Ech_0284	Ech_0379	Ech_0480	Ech_0490	Ech_0601	Ech_0660	Ech_0760
Infection persistence	-	-	+	-	+	+	+	-	+

The signs + and – referrer to positive or negative for the mutants in the animal samples assessed.

Dog infection data#: Mutants assessed in blood of animals infected as pool of mutants or as clonal population

Mutant #	Ech_0202	Ech_0230	Ech_0284	Ech_0379+	Ech_0480	Ech_0490	Ech_0601	Ech_0660+	Ech_0760
Infection persistence	+	-	+	-	+	+	+	-	+

*Deer infections were assessed up to 62 days; #Dog infections were assessed up to 44 days; +Ech_0379 and Ech_0660 mutants were detected in dogs on day 2 post-infection when assessed as clonal population

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PUBLICATIONS DURING THE FUNDING PERIOD: The grant support resulted so far in 10 peer-reviewed research publications; one submitted (to Molecular Microbiology) and several more in preparation. We also published 8 reviews/book chapters. The funding was instrumental in generating and disseminating considerable scientific knowledge in support of advancing the understanding of tick-borne rickettsial diseases. The progress report publications list is provided after the references section.

RESEARCH DESIGN AND METHODS: We believe that the novel data from the funded R01 project, the investigators' expertise and our resources are the primary strengths of our proposal. The goals are to continue research in assessing the importance of differential pathogen gene expression in vertebrate hosts and for tick transmission of *E. chaffeensis* and how differential expression contributes to the pathogen's *in vivo* growth in dual host environments. We believe that the progress we made and the goals we set for the competing renewal application are important in understanding pathogenesis and stimulating similar research in other closely related pathogenic organisms.

Specific Aim 1: Characterize *E. chaffeensis* RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression:

Sub aim 1) Mapping -35 and -10 motifs of promoters and binding domains of transcription factors, sigma 70 and sigma 32.

Significance and innovation: To the best of our knowledge, we are the first and the only research group investigating the gene regulation in *Ehrlichia* species. We have made significant progress in identifying genes recognized by the primary sigma factor, σ70, and by the only alternative sigma factor, σ32. Methods useful in understanding the regulation of gene expression in *E. chaffeensis* are important as studying the regulation of gene expression *in vivo* in intraphagosomal pathogens is not possible at this time. This remains a challenge despite the considerable progress made by us in creating mutations in this organism. The RNAP function may be crucial in supporting *E. chaffeensis* adaptation to different host cell environments and for its conversion from the dense core (infectious form) to the reticulate bodies (replicating form) and then back to dense core form within an infected host cell. Based on the data demonstrating the shared recognition of *E. chaffeensis* promoters by RNAP holoenzyme containing either σ32 or σ70 and in transcribing the gene products Unpublished submitted Unpublished, our hypothesis is that *E. chaffeensis* gene regulation is complex and its transcription system is evolved to better serve the needs of the pathogen's growth under the stressful environment of vertebrate and tick hosts. The innovative research proposed in this aim, focusing on further understanding the regulation of gene expression, is critical to defining how the pathogen senses and alters its gene expression in support of completing transmission cycle in vertebrate and tick hosts.

Approach: During the previous funding cycle, we prepared RNAP holoenzyme containing *E. chaffeensis* sigma factors, σ32 or σ70, coupled with *E. coli* core enzyme for use in mapping the pathogen genes. We developed two approaches to map the transcription machinery of *E. chaffeensis*; *in vitro* transcription system (with σ32 or σ70) and transcription mapping using *E. coli* as the surrogate (for σ32 only). For *in vitro* transcription system, we utilized the purified native RNAP or used the recombinant *E. chaffeensis* sigma factors; σ32 or σ70 reconstituted with *E. coli* core enzyme. In *E. coli* surrogate system, we developed a method to map *E. chaffeensis* σ32 gene promoters using *E. coli* mutant not expressing the native σ32. As we do not see any obvious differences when using the purified native holoenzyme or recombinant σ70-reconstituted holoenzyme, our preference in this project will be to use the recombinant protein reconstituted RNAP holoenzyme. This system will be clean and will aid in clearly distinguishing the transcription with RNAP containing σ32 or σ70. The *in vitro* transcription is an ideal system for mapping the regulators of gene expression [71]. For mapping the promoter regions to identify RNAP binding motifs, the better approach would be to use the *E. coli* system expressing *E. chaffeensis* σ32 or σ70. In the preliminary studies, we presented the mapping of the -35 motif (by deleting all six nucleotides) of *dnaK* promoter and its interacting amino acids in region 4.2 of *E. chaffeensis* σ32 were mapped. These studies will be extended for mapping precise binding sites of the -35 and -10 motif of *dnaK* promoter and the σ32 interacting amino acids. To evaluate if minor differences in the -35 and -10 motifs in different gene promoters contribute to RNAP specificities, we will investigate another σ32 promoter, *groE*. Contributions of all six nucleotides of each motif will also be assessed by point mutation analysis at each position of the motifs (since our last submission, we made progress in preparing numerous point mutation constructs in support of this experiment).

Similar studies to map *E. chaffeensis* σ70 in *E. coli* requires the development of a modified *E. coli* strain where its native σ70 is mutated and is complemented by the *E. chaffeensis* σ70. We propose two approaches to execute this goal; 1) replace *E. coli* σ70 with the *E. chaffeensis* σ70 by utilizing the well-established red recombinase system [26,72] (Addgene, Cambridge, MA). This work is already underway; a chimeric segment was prepared containing 50 bp long sequences each of the 5' and 3' end regions upstream or downstream to *E. coli* RpoD gene coding regions, but missing its entire coding region and is replaced with the *E. chaffeensis* RpoD coding sequence. This segment will be used to create a replacement mutation in *E. coli* with the aid of a red recombinase system. As there is always a risk for failure to recover a viable *E. coli* strain, we planned an

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alternate approach; we obtained an *E. coli* strain in which the native RpoD gene is under the control of the repressible *trp* promoter (*E. coli* strain, CAG20177; it is a generous gift from Dr. Carol Gross at University of California, San Francisco, CA) [73]. *E. chaffeensis* RpoD gene in a plasmid and a compatible plasmid containing the *E. chaffeensis* σ70 promoter (p28-Omp 14 and 19 gene promoters) will be transformed into this *E. coli* strain and following repression of native σ70, promoter studies will be performed similar to our description of σ32 promoters. Together, the two independent approaches will aid in mapping the RNAP binding motifs and interacting amino acids of *E. chaffeensis* σ70.

Expected outcomes, potential pitfalls and alternative strategies: The goals to map four promoters (two each of σ32 and σ70 genes) will allow us to define how *E. chaffeensis* RNAP holoenzyme with σ32 and σ70 functions in altering its gene expression. We are adept in performing all the proposed experiments. The experimental protocols are very similar to those utilized in the experiments performed during the previous funding cycle. We have a strong team having hands on experience in executing all of the experiments proposed. Alternative strategies are already thought-out and integrated in the experimental design to ensure success. We believe that we are well-prepared to overcome any unforeseen challenges in executing the goals.

Sub aim 2) Identify factors (regulatory proteins) contributing to the host-specific differential gene expression

Significance and innovation: The results from the first experiment will aid in defining unique regulatory machinery used by *E. chaffeensis.* This sub aim will address how *E. chaffeensis* regulates gene expression in response to its growth in vertebrate and tick cell environments; we will prepare two cell lysates of *E. chaffeensis* grown in macrophages and tick cells. The lysates are expected to contain regulatory proteins interacting with the promoters of genes under investigation; we will focus on four genes [two genes each transcribed with the aid of σ32 (*dnaK* and *groE*) and σ70 (p28-Omp genes 14 and 19)]. The competing renewal will focus to identify regulators influencing the gene expression (positively or negatively).

Approach: The goal of this experiment is to assess variations in gene expression from the above listed four genes under *in vitro* conditions in the presence of *E. chaffeensis* lysates derived from macrophages or tick cells. This is to test the hypothesis that transcription from *E. chaffeensis* is varied because one or more regulators bind to DNA elements in a promoter to alter gene expression. *E. chaffeensis* lysates will be prepared as described earlier [38]. Varying concentrations of lysates will be added and rates of transcription will be measured. The binding of regulatory proteins to specific motifs of the gene promoters will be assessed by gel mobility shift and DNase I foot printing assays. *In vitro* transcription assays will be performed using RNAP holoenzyme containing recombinant σ32 or σ70. To confirm the specific binding of regulators and their effects on transcription, the assays will be repeated with deletion constructs or constructs containing specific mutations in the promoter regions. Further, the assays will include appropriate controls such as cold competitor DNA segments or non-specific DNA. DNase I foot printing assays will be done to determine the exact location of sequence to which DNA binding protein interactions occur [74]. The assays will be performed as described above for gel mobility shift assays, except that the DNA will be end-labeled with a tag at the 5' end and used for DNase I digestion. This technique is a simple conjoining of the Maxam-Gilbert DNA-sequencing method for DNase-protected fragment isolation [74]. The assays will be performed with kits commercially available (Amersham Pharmacia Biotech). Gene-specific transcription regulators involved in the differential regulation will be identified by mass-spectrometry analysis. Proteins bound to specific DNA regions will be recovered from polyacrylamide gels or by utilizing affinity chromatography and subjected to LC-MS/MS analysis, as we performed earlier [29-31]. In support of the goals of this sub aim, we recombinantly expressed and purified five predicted DNA binding proteins/transcription regulators of genes identified in the *E. chaffeensis* genome and are now performing experiments to determine if one or more of these proteins are involved in differential gene expression (targeted approach). Indeed, we found one of the proteins, Ech_0795, a homolog of *ApxR* of *A. phagocytophilum* which is involved in differential gene expression of membrane proteins [62], to be specifically binding to the promoter segments of p29-Omp 14 and 19 genes (Figure 5). This targeted approach in identifying regulatory proteins compliments our goals in determining transcription regulators involved in differential gene expression.

Expected outcomes, potential pitfalls and alternative strategies: The experiments involve the preparation of *E. chaffeensis* lysates, gel mobility shift, DNase I foot printing assays, deletion/mutational analysis and LC-MS/MS analysis. We have previously used all of the protocols with the exception of DNase foot printing. This assay will be performed using a commercial kit. We have considerable molecular biology expertise and are optimistic that we can troubleshoot any potential problems that may arise in executing the goals. This sub-aim will allow us to identify unique host-specific differences in gene regulation and will aid in identifying transcription regulators contributing to the differential regulation. This research will open many new opportunities to understand the differential gene regulation by *Ehrlichia* species in a host cell-specific manner and the studies will have important implications devising methods of controlling infections with *E. chaffeensis* and other related pathogens in people.

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Specific Aim 2) Evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth

Significance and innovation: Our mutagenesis and infection screening experiments, described in progress report (Specific aim 4), led to the identification of three differentially expressed genes of *E. chaffeensis* (Ech_0230, Ech_0379 and Ech_0660) required for the pathogen's growth *in vivo*. Our bioinformatics analysis suggested that these genes encode for membrane associated proteins [42]. The focus of this aim is to map their expression pattern and to assess if the mutants or recombinantly expressed proteins from these three genes are suitable as immunogens to confer protection against wild-type infection challenge. This will be the first attempt in assessing the functional significance of gene products found to be important for the pathogen's *in vivo* growth. This will also be the first study in evaluating the basic research outcomes for their value in translational research (second part of this aim).

Approach: Two experiments will be performed in this aim; 1) map the expression profiles of the three genes and 2) assess the value of the three gene mutant clones and their gene products in conferring protection against infection challenge.

Experiment 1) Transcriptional analysis to map the RNA expression will be performed by real-time RT-PCR similar to the methods we described in one of our previous studies [35] using RNA isolated from different time points post inoculation of the wild-type culture-derived organisms into macrophage and tick cell cultures. To map the protein expression, we will first make the recombinant proteins and use them to prepare polyclonal sera; we will use pET28 plasmid and the *E. coli* strain, BL21 (DE3) (Novagen, Billerica, MA), to express and purify the recombinant proteins from the genes Ech_0230, Ech_0379 and Ech_0660. The molecular methods are routinely performed and we have the expertise to troubleshoot technical challenges. The purified recombinant proteins will then be used to prepare polyclonal sera from a commercial vendor (two rabbits per antigen will be used as per the approved animal use and handling protocol; letters attached). To map the localization of the three gene products, we will conduct two independent experiments; a) map the protein expression and localization in subcellular fractions: *E. chaffeensis* infected host cell cultures (vertebrate macrophages or tick cells) will be recovered from different time points post infection (6 h to 96 h) and will be used to fractionate the proteins as the *Ehrlichia* lysate supernatant, *Ehrlichia* membrane fraction, host cell cytoplasmic fraction, and host cell nuclear fraction. The protein fractions will be resolved on a polyacrylamide gel and subjected to Western blot analysis; b) perform the protein localization experiments in infected host cells, also following different times post infecting the host cells by subjecting to fluorescent marker tagged secondary antibodies to recognize the gene products of Ech_0230, Ech_0379 and Ech_0660. These two independent experiments will allow us to map the protein expressions relative to time post infection and aid in identifying *Ehrlichia* form (dense core or reticulate form or in both) where the proteins are expressed and whether or not the proteins are expressed on bacterial membrane or phagosomal membrane or secreted into host cytoplasm or transported to other organelles of the host cell.

Experiment 2) To examine if one or more of the three gene mutant clones and their gene products confer protection against infection challenge requires the generation of clonal population of mutants, availability of sufficient quantity of antigens and performing infection studies. We already purified clonal populations of all nine transposon mutants which included the Ech_0230, Ech_0379 and Ech_0660; clonal isolation of mutants was accomplished by serial dilution cloning method {briefly discussed in [42]}. Animal infections will be performed as we described above in the progress report. To evaluate the mutants as immunogens, five dogs each will be infected with clonal mutants, infections will be monitored for three weeks by culture and nested PCR analysis (we expect that the animals will be negative; our new preliminary data support this hypothesis), the animals will be boosted with a second inoculum and followed for three additional weeks prior to challenging with the wild-type *E. chaffeensis* strain (Arkansas) and observing infection progression and antibody responses. The experiment will include two animals as a control group which will be infected with wild-type *E. chaffeensis*. As the animals represent outbred population, variations in infection progression and host responses are likely (similar to our prior observations; preliminary data). Therefore, to draw meaningful conclusions, we feel that it is necessary to perform infections at least in five dogs each. To reduce the animal to animal variation, we will also attempt to have a group of animals from the same litter where possible. A complementary experiment is to assess if the recombinant Ech_0230, Ech_0379 and Ech_0660 gene products also confer protection. The primary and secondary immunogen regimens and challenge infections schedule will be followed similarly as proposed for assessing the live mutant clones and by involving five animals per antigen. For this experiment, the control dogs will be inoculated with heat inactivated *E. chaffeensis* organisms. As this will be the first such experiment for using *E. chaffeensis* and dogs, it is entirely unclear what should be the ideal quantity of antigen and what adjuvant works the best. Therefore, we opted to follow the protocols reported with promising results for another vaccine study in dogs [75].

We performed a pilot study in assessing the Ech_0660 mutant in generating host response in deer and in conferring protection against wild-type infection challenge; the results are promising. The mutant did not grow for the 4 weeks of assessment following its infection into three animals, whereas wild-type and

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Ech_0284 mutant clone persisted (Table 2). IgG response in animals infected Ech_0660 mutant clone is very similar to that observed for wild-type infected or Ech_0284 infected animals (ELISA data for wild-type and Ech_0660 mutant were presented in **Figure 7).** Ech_0660 mutant infected animals were challenged with wild-type *E. chaffeensis* at day 31 post-infection and the animals were monitored for the infection and for IgG responses for another 44 days (**Table 2** and **Figure 7**). The Ech_0660 mutant infected animals remained PCR and culture negative for the organism, but continued to exhibit IgG response against *E. chaffeensis* antigens. Considering these exciting preliminary data, we expect the anticipated outcomes for the experiments proposed in this aim.

Table 2: Assessing Ech_0660 mutant in conferring protection against wild-type *E. chaffeensis* challenge

	*Days post infection**																				
	0	3	5	8	10	14	21	23	28	31	35	42	49	53	55	58	61	65	68	72	75
Uninfected	-	-	-	-	-	-	-	-	-	-	Ech_0660 infected animals were challenged with wild-type *E. chaffeensis* on day 31										
Wild-type	-	-	+	+	+	+	+	+	+	+											
Ech_0284	-	-	+	+	+	+	+	-	-	-											
Ech_0284	-	-	-	+	+	+	+	+	-	+											
Ech_0660	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ech_0660	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ech_0660	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Infection in animals was assessed by culture recovery or nested PCR analysis of blood samples

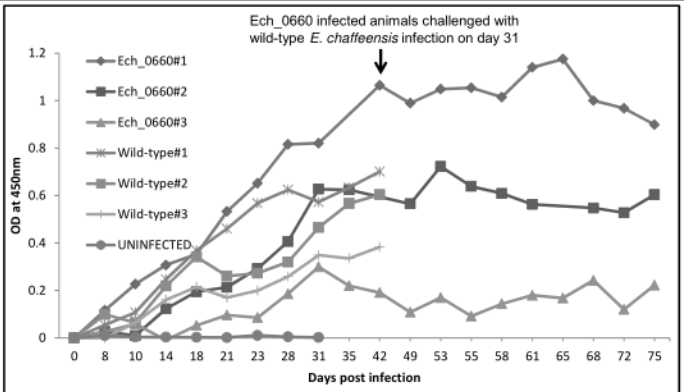

Figure 7: IgG response assessed by ELISA against whole cell *E. chaffeensis* antigens in deer infected with wild-type or Ech_0660 mutant clones.

Expected outcomes, potential pitfalls and alternative strategies: The studies will aid in defining the protein expression. Based on the bioinformatics analysis, proteins made from the three genes with mutations are likely membrane bound proteins. We presume that the proteins may localize on the bacterial membrane or may be transported into the phagosomal membrane. One of these proteins (Ech_0379 product) is predicted as a Na^+/H^+ antiporter protein. We would like to further investigate the functions and to assess biological significance of these three gene products. These experiments, however, cannot be initiated until the basic information about the proteins is critically assessed (the focus of this aim). The data from these experiments will allow us to determine the expression pattern, localization and the contributions of host cell environments. We anticipate generating meaningful data from experiment 2 regarding the vaccine potential of mutants and recombinant proteins made from genes identified as critical for *in vivo* growth. The outcomes will have implications for the vaccine development in the near future. We, therefore, expect to generate significant novel data in advancing our understanding of the proteins and their importance to *in vivo* infection, as well as to assess the potential of the *in vivo* infection-specific proteins and attenuated mutants for the development of novel therapeutics and prevention methods.

Specific Aim 3: Perform mutational analysis and *in vivo* screening to identify genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts

Significance and innovation: To understand the mechanisms by which bacterial pathogens overcome the immune system in causing diseases, it is essential to identify pathogen proteins needed for each stage of the infection process. Identification of many important pathogenic proteins *in vitro* is often not possible, as many contributing host factors cannot be reproduced in a laboratory. The availability of a genetic manipulation system significantly aids in mapping proteins critical for *E. chaffeensis* survival and pathogenicity. We established the mutagenesis and screening methods useful to identify genes essential for *in vivo* survival. This *in vivo* negative selection screen is a very powerful method for mapping genes important for pathogenesis and is reported for several pathogenic bacteria [51,76-78]. To the best of our knowledge, we are the first in developing such a method and have shown the proof of principle by identifying essential genes of an obligate intracellular pathogen. The primary focus of this aim is to expand this approach by screening approximately 20% of the genome to identify genes essential for the bacterial growth *in vivo*. This is the first study in an *Ehrlichia* species to identify specific genomic regions needed for the *in vivo* infection.

Approach: We will generate large pools of *E. chaffeensis* transposon mutants by performing the experiments as outlined in the progress report. We propose to perform about 40 transposon mutagenesis experiments; on average one experiment will yield 5 mutants and based on this assumption, we estimate to have as many as

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200 mutations in *E. chaffeensis*. These may represent mutations mostly at unique genomic locations, although some of them may be located within the close proximity to another mutation. We will assess the mutations by Southern blot analysis of the genomic DNA collected from the mutant populations and create a pool containing approximately 10 mutants (about two transformations). The pools will then be used to infect three dogs each. Infections in the animals will then be monitored for 8 weeks; blood samples will be collected twice a week and infection will be assessed by nested PCR and culture recovery methods. The pathogen DNA from input pool and output pool will be assessed by mapping the transposon insertions and those missing during the *in vivo* growth will be identified. We consider two independent methods for mapping the transposon mutation sites; 1) amplify a segment using insertion-specific primer and with a random primer by following the semi random nested PCR and sequencing [42], or 2) by performing microarray analysis. The first approach, while we have the expertise in executing it, can be labor intensive for mapping large pool of mutants. The second method can be much more rapid and efficient; microarray chip spanning the entire *E. chaffeensis* genome will be prepared at a commercial vendor and utilized to map the mutation sites. Whole genome sequencing may be another rapid method for consideration. Considering the recent studies suggesting the significant improvements to the whole genome sequencing and reduced cost for this method make it an attractive alternate strategy [79]. In the event we choose whole genome sequencing approach, we will use a fee-for-service facility such as at (https://gtac.wustl.edu/services/sequencing/) Washington University at St. Louis, MO. Importantly, we believe that considering the rapid advances of technology and our own capabilities, mapping the genomic transposon insertion sites will be an easily achievable goal.

We propose to infect a pool of approximately 10 mutants in three independent animals and the infection status will be assessed twice a week for two months. The analysis represents a total of about 50 blood samples assessed per each pool. For xenodiagnoses, nymphal ticks (typically, we attach 250 nymphs) will be allowed to acquisition feed on animals starting from day 5 post infection. Our prior data when assessed in deer suggested that the infection rates in ticks reach up to 70-90% with the pathogen when employing this strategy. It is not clear if the same infection rates will be observed similarly in dogs. We will evaluate a minimum of 20 ticks from each animal following the molting to adult stage to assess which mutants are acquired by ticks. In the event the infection rates are low, we will increase the ticks assessed to 40 or more. We have experience in conducting tick acquisition feeding experiments using uninfected ticks and naïve dogs. The tick recovery of the fed nymphs dropped off and molted was about 60-70% (unpublished work). Thus, we do not anticipate any challenges in executing this experiment. Together, the assessments of blood by PCR and culture, and xenodiagnoses will aid us to determine which genomic regions of *E. chaffeensis* are critical for the *in vivo* growth.

One possibility for the lack of growth of some mutants *in vivo* is a likely difference in mutant growth rates. To rule out this possibility, we will repeat the infection experiment on the mutants identified as attenuated *in vivo* in the first experiment {based on our prior published data, we estimate that the growth of approximately 30% clones will be impacted (about 60 mutants total)}. We will clonally purify these mutants, randomly select pools of 5 each and use them to repeat the infections in two animals per pool. Similarly, we will select 10 most promising candidates (as we define as those containing mutations in likely membrane protein or predicted secreted protein genes) and perform infection experiments individually. Infection assessments will be followed similarly as in the first experiment by sampling blood collected over a two month period and examine for the presence of organisms by PCR and *in vitro* cultivation. Taken together, we will be able to unequivocally identify mutants truly attenuated as a result of gene disruptions.

Expected outcomes, potential pitfalls and alternative strategies: Disruption of genes in the *E. chaffeensis* genome and their assessment in dogs and ticks by screening many pools of mutants is an innovative goal aids in identifying large numbers of genes critical for the *in vivo* growth. As we propose to assess mutations in nearly 200 genomic sites, we expect to identify about 50-60 essential genes. Even if this number is significantly low, say 20 genes, the outcomes will still have enormous implications in advancing our understanding of the pathogenesis of *E. chaffeensis*. This will lead to the identification of many genes essential for the pathogenesis and also lead to the development of many attenuated strains. The project involves a very effective and productive collaboration among scientists having high level of demonstrated expertise from two academic institutions. We believe that we can overcome any unforeseen technical challenges during the course of this exciting project. We are optimistic that the experiments will be completed with no potential challenges, as we employ the same methods as described in the progress report.

TIME FRAME: The major emphasis in years 1 and 2 will be on Aim 1 and 2. Aim 1 will continue through years 2 to 5. The majority of the work on Aim 3 will be performed in years 3 to 5. Dog infections for Aim 2 will be executed in years 1 and 2, whereas infection experiments planned in Aim 3 will be carried out in years 3 to 5.

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PROGRESS REPORT PUBLICATION LIST:

Research papers published:

1) Total, membrane, and immunogenic proteomes of macrophage- and tick cell-derived *Ehrlichia chaffeensis* evaluated by LC-MS/MS and MALDI-TOF methods. Seo GM, Cheng C, Tomich J, Ganta RR. *Infect Immun*. Infect Immun. 2008, 76:4823-32. PMID: 18710870, PMCID: PMC2573352
2) Promoter analysis of macrophage- and tick cell-specific differentially expressed *Ehrlichia chaffeensis* p28-Omp genes. Peddireddi L, Cheng C, Ganta RR. BMC Microbiol. 2009 May 19;9:99. PMID: 19454021, PMCID: PMC2694197
3) Comparison of iatrogenic *Anaplasma marginale* transmission by needle and needle-free injection techniques J. B. Reinbold, J. F. Coetzee, L. C. Hollis, J. S. Nickell, C. Riegel, J. C. Huff, and R. R. Ganta. Am J Vet Res. 2010 Oct;71(10):1178-88. PMID: 20919904
4) Detection of *Anaplasma marginale* and *A. phagocytophilum* in bovine peripheral blood samples by duplex real-time reverse transcriptase PCR assay. Reinbold JB, Coetzee JF, Sirigireddy KR, Ganta RR. J Clin Microbiol. 2010 Jul;48(7):2424-32. Epub 2010 May 12. PMID: 20463162, PMCID: PMC2897487
5) The efficacy of three chlortetracycline regimens in the treatment of persistent *Anaplasma marginale* infection. Reinbold JB, Coetzee JF, Hollis LC, Nickell JS, Riegel C, Olson KC, Ganta RR. Vet Microbiol. 2010 Sep 28;145(1-2):69-75. Epub 2010 Mar 3. PMID: 20346598
6) Isolation and characterization of Ehrlichia chaffeensis RNA polymerase and its use in evaluating p28 outer membrane protein gene promoters. Bonto Faburay, Huitao Liu , Lalitha Peddireddi and Roman R Ganta. BMC Microbiology 2011, 11:83doi:10.1186/1471-2180-11-83 PMID:21513529, PMCID: PMC3108270
7) Transmission Electron Microscopy Reveals Distinct Macrophage- and Tick Cell-Specific Morphological Stages of *Ehrlichia chaffeensis.* S. E. DeDdonder, C. Cheng, L. H. Willard, D. L. Boyle, and R. R. Ganta. PLoS One. 2012; 7(5): e36749. Published online 2012 May 15. doi: 10.1371/journal.pone.0036749 PMID:22615806, PMCID: PMC3352939
8) Identification of critical host mitochondrion-associated genes during Ehrlichia chaffeensis infections. Von Ohlen T, Luce-Fedrow A, Ortega MT, Ganta RR, Chapes SK. Infect Immun. 2012 Oct;80(10):3576-86. doi: 10.1128/IAI.00670-12. Epub 2012 Jul 30. PMID: 22851751, PMCID: PMC3457586
9) Targeted and Random Mutagenesis of *Ehrlichia chaffeensis* for the Identification of Genes Required for In vivo Infection. Cheng C., Nair A. D. S., Indukuri V. V, Gong S., Felsheim R. F., Jaworski D., Munderloh U. G. and Ganta R. R. PLoS Pathog. 2013 Feb;9(2):e1003171. doi: 10.1371/journal.ppat.1003171. Epub 2013 Feb 14. PMID: 23459099, PMCID: PMC3573109
10) Aggregate-Reactivation Activity of the Molecular Chaperone ClpB from Ehrlichia chaffeensis. Zhang T., Kedzierska-Mieszkowska S., Liu H., Cheng C., Ganta R. R., Zolkiewski M. PLoS One. 2013 May 7;8(5):e62454. doi: 10.1371/journal.pone.0062454. PMID: 23667479, PMCID: PMC3646808

Research papers submitted/in progress:

1) Unpublished
2) Unpublished
3) Unpublished

Reviews and book chapters-published:

1) Cheng C, Sirigireddy KR, Ganta RR. Isolation and molecular detection of *Ehrlichia* from vertebrate animals. *Curr Protoc Microbiol*. 2008 May;Chapter 3:Unit 3A.3. PMID: 18770538
2) Cheng C, Ganta RR. Laboratory maintenance of *Ehrlichia chaffeensis* and *Ehrlichia canis* and recovery of organisms for molecular biology and proteomics studies. *Curr Protoc Microbiol*. 2008 May;Chapter 3:Unit 3A.1. PMID: 18770537
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5) R. R. Ganta. Book Chapter: 'Chlamydiaceae' in 'Veterinary Microbiology, Third Edition' (Wiley-Blackwell Publishing, 2013). Edited by D. S. McVey, M. Kennedy and M. M. Chengappa. ISBN: 978-0-470-95949-7
6) R. R. Ganta. Book Chapter: 'Rickettsiaceae: *Rickettsia* and *Coxiella* species' in 'Veterinary Microbiology, Third Edition' (Wiley-Blackwell Publishing, 2013). Edited by D. S. McVey, M. Kennedy and M. M. Chengappa. ISBN: 978-0-470-95949-7
7) R. R. Ganta. Book Chapter: 'Anaplasmataceae: *Anaplasma* species' in 'Veterinary Microbiology, Third Edition' (Wiley-Blackwell Publishing, 2013). Edited by D. S. McVey, M. Kennedy and M. M. Chengappa. ISBN: 978-0-470-95949-7
8) R. R. Ganta. Book Chapter: 'Anaplasmataceae: *Ehrlichia* and *Neorickettsia* species' in 'Veterinary Microbiology, Third Edition' (Wiley-Blackwell Publishing, 2013). Edited by D. S. McVey, M. Kennedy and M. M. Chengappa. ISBN: 978-0-470-95949-7

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VERTEBRATE ANIMALS: In this study, we will be using specific pathogen free dogs. Dogs will be used for experiments proposed in aims 2 and 3. In aim 2, the two complete experiments for each of the three genes will require 5 animals for each clonal mutant population and another group of five animals each for each recombinant antigen (total of 30 animals). In addition, we will be using four animals that will serve as controls (two for wild-type infection control group and two for inactivated whole cell antigen control group). For experiments proposed in aim 3, we will use 3 animals for each pool of 10 transposon mutants. Since we proposed to evaluate 200 mutants, the total number of animals required will be 60 for the first experiment of Aim 3. Second part of aim 3 will require 44 animals (12 pools of mutants will be assessed using two animals each and 10 individual mutants will be assessed by infecting in two animals each which totals 44 animals). Together, total dogs to be used in these experiments will be 138. A total of 6 rabbits will also be used for preparing the polyclonal sera made against three different antigens (in support of Aim 2). The antibody preparation will be performed at a commercial vendor as per the protocol approved by the NIH (letter attached). Vertebrate animal care for dogs is outlined below.

Vertebrate Animals Statement:

1. The proposed research utilizes dogs as hosts for bacterial pathogenesis studies and tick acquisition/transmission studies.

2. *Ehrlichia chaffeensis*, the intracellular bacteria that cause monocytic ehrlichiosis is accidentally acquired by dogs (similar to humans). We have developed a dog model for these studies. Specific pathogen-free beagle breed dogs are readily available from commercial vendors. We will require 138 dogs to accomplish the goals of experiments as proposed.

3. Daily care for the dogs will be managed by the Laboratory Animal Resource staff. Dogs are offered fresh water and feed ad lib and monitored by attending animal care personnel twice daily. Veterinary care which includes daily cleaning of cages and observing animals for normal behavior. The animal care group at Kansas State University includes several attending veterinarians and trained animal care workers, all of whom are supervised by the Director of the Laboratory Animal Resources. Animal care is also available when needed from the K-State Veterinary Hospital.

4. Dogs will be subjected to venipuncture, venous injections of bacteria and immunizations of antigens that cause only momentary discomfort. Blood (about 5 cc per sample) will be collected twice weekly, while bacterial injections and immunizations (as outlined in the proposal) will be done less frequently. Infected animals will be monitored for the symptoms of infection. Symptoms in dogs are generally mild and rarely complicated by serious outcomes. Immunizations will be done using standard procedures and an adjuvant approved by the University Institutional Animal Care and Use Committee (IACUC).

5. Euthanasia will be performed in accordance with University IACUC recommendations that are consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

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Thermo SCIENTIFIC

Dear Sir or Madam:

Thermo Scientific Pierce Biotechnology products rabbit immunization protocols and animal handling procedures are approved by NIH, USDA and ALAAAC through Strategic Diagnostics and their animal facility located in Wyndham, Maine. Every protocol is submitted for internal review by the IACUC committee, which makes recommendations and assures that the use of animals is justified and that animals are cared for properly.

A staff of trained husbandry personnel supports the daily activities of feeding, cleaning, and routine treatments of animals. Animals are observed daily for condition and appetite by husbandry personnel, and veterinarians monitor all reporting.

The Animal Care and Use Committee (IACUC) has one veterinarian, three members that are husbandry staff, and one member that is a non-affiliated community member. AAALAC inspection is carried out at least once every two years and status reports are submitted yearly to the USDA and NIH.

NIH (OLAW) assurance number:	A3975-01 exp. 3/16
USDA registration number:	50-R-0013 exp. 7/16/14
AAALAC accreditation number:	001011 exp. 11/15
PHS assurance number:	A3975-01 exp. 3/31/16

Detailed protocol approvals by IACUC can be provided upon request.

Sincerely,

Steven M. Franklin, M.S.
Manager, Custom Antibodies and Proteins
Thermo Scientific Pierce Protein Research

Thermo Fisher Scientific
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Rockford, IL. 61101
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DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:
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Fax: (301) 915-9472

March 8, 2012

Jeffery K. Thompson
Redacted by agreement

Dear Mr. Thompson:

The Office of Laboratory Animal Welfare (OLAW) has reviewed and approved the Animal Welfare Assurance (Assurance) which was submitted in compliance with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy) revised August 2002.

Your renewed Assurance with identification **#A3975-01** became effective on March 8, 2012 and supersedes any previously issued Assurance. The approval period is for four years, and will expire on March 31, 2016. Please be sure to reference your Assurance number in all correspondence to this Office.

The Assurance is a key document in defining the relationship of your Institution to the PHS since it sets forth the responsibilities and procedures of your Institution regarding the care and use of laboratory animals. Among the important elements of the Assurance, I would especially call your attention to the reporting requirements which are essential for continued compliance with the PHS Policy.

Please note that an Annual Report to OLAW is required at least once every 12 months. The reporting period is the calendar year. Reports for the previous calendar year are due **January 31.** Therefore your Annual Report for 2012 is due to this office by January 31, 2013.

A copy of the approved Assurance signature page is enclosed. If I may be of any further assistance, please contact me.

Sincerely,

Personal Info

Brent C. Morse, D.V.M.
Animal Welfare Program Specialist
Office of Laboratory Animal Welfare

cc: Michael Hall, IACUC Chair (Maine site)
Dale Onisk, Ph.D., IACUC Chair (Delaware site)

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aaalac INTERNATIONAL

Association for Assessment and Accreditation of Laboratory Animal Care International

5283 Corporate Drive, Suite 203
Frederick, MD 21703-2879

November 29, 2012

Jeffrey K. Thompson, A.S., L.A.T.
Facility Director and Institutional Official
Redacted by agreement

Dear Mr. Thompson:

The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International) is pleased to convey that Redacted by agreement is accredited. Our records show that Redacted by agreement initially achieved AAALAC International accreditation on October 20, 2000.

This interest, contribution and participation in the AAALAC International accreditation program is valued and appreciated. As you are aware, AAALAC International conducts site visits to institutions and requires being apprised of significant programmatic changes during the interim between these site visits. Should you desire additional information or wish to comment on any aspect of the accreditation process, please contact our office.

Sincerely,

Personal Info

Kathryn A. Bayne, M.S., Ph.D., D.V.M., DACLAM, CAAB
Global Director

KAB: cmf
001011

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RE: IACUC Approvals

To Whom It May Concern:

On 06/26/12 the Institutional Animal Care and Use Committee (IACUC) for Redact formally approved polyclonal antibody production in several species. These species include Rabbits, Goats, Sheep, Chickens, Mice, Rats and Guinea Pigs. The Standard Protocols used by Redac for these respective species are covered by this approval. The approval for all Standard Protocols is made minimally on an annual basis and all Standard Protocols expires yearly.

Approval numbers are as follows:

Rabbits:	R-R062612
Goats:	R-GS062612
Sheep:	R-GS062612
Guinea Pig:	R-P062612
Rat:	R-MR062612
Mouse:	R-MR062612
Chicken:	R-C062612

All project submissions are reviewed by the IACUC to insure that they are within the guidelines of the Animal Welfare Act and "*The Guide for the Care and Use of Laboratory Animals*".

Regards,

Michael Hall

Personal Info

IACUC Chairperson

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Redacted by agreement

July 16th, 2013

Roman Ganta, M.S., Ph.D.
Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine
Kansas State University
800 Denison Ave
Manhattan, KS 66506

Dear Roman,

It is my pleasure to serve as a consultant on your competing renewal application titled 'Vector and host contributions to the regulation of *E. chaffeensis* gene expression'. I enjoyed discussing in detail and learn about the exciting goals you set for the proposed research. I felt that the research plan is well thought-out, very worthwhile and exciting to pursue. I am happy that the team approach was successful in developing and characterizing stable transposon mutants, and in assessing the mutants to identify genes important for in vivo infection of *E. chaffeensis*. Congratulations on having this research published in PLoS Pathogens, a clear demonstration that our collaboration is very effective in promoting good science.

This is to further confirm that I will continue to offer support for your plan to develop a transposon mutant library. In particular, I am happy to be named as consultant on the project and look forward to providing advice in support of the proposed goals of specific aim 3. As always, you are most welcome to contact me any time. The team approach you embrace is important for advancing research on severely under-studied pathogens such as *E. chaffeensis.*

With best personal regards,

Personal Info

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RESOURCE SHARING PLAN

The PI acknowledges that NIH considers the sharing of unique research resources developed through NIH-sponsored research an important means to enhance the value and further the advancement of the research. When resources have been developed with NIH funds and the associated research findings published or provided to NIH, the findings will be made readily available for research purposes to qualified individuals within the scientific community.

This R01 Renewal Application does not require the following specific sharing plans:

1) Data Sharing Plan- The project does not seek $500,000 or more in direct costs (exclusive of consortium F&A) in any year.

2) Sharing Model Organisms- The project does not anticipate the development of model organisms.

3) Genome Wide association Studies- The project does not seek funding for a genome-wide association study.

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PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☐ New ☒ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier: AI070908

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☒

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

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4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No

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Notice of Award

RESEARCH **Federal Award Date:** 07/20/2015

Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI070908-07
FAIN: R01AI070908

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Mr. Lowe, Paul R.
Asst Vice President/Research
2 Fairchild Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Period Of Performance:
Budget Period: 08/01/2015 – 07/31/2016
Project Period: 08/01/2006 – 07/31/2018

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $441,044 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI070908. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

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Laura C. Eisenman
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

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SECTION I – AWARD DATA – 5R01AI070908-07

Award Calculation (U.S. Dollars)

Salaries and Wages	$139,958
Fringe Benefits	$40,858
Personnel Costs (Subtotal)	$180,816
Supplies	$87,425
Travel Costs	$9,801
Other Costs	$15,987
Federal Direct Costs	$294,029
Federal F&A Costs	$147,015
Approved Budget	$441,044
Total Amount of Federal Funds Obligated (Federal Share)	$441,044
TOTAL FEDERAL AWARD AMOUNT	$441,044
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$441,044

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
7	$441,044	$441,044
8	$441,044	$441,044
9	$441,044	$441,044

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy, Immunology and Transplantation Research
CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908C
PMS Account Type:	P (Subaccount)
Fiscal Year:	2015

IC	CAN	2015	2016	2017
AI	8472302	$441,044	$441,044	$441,044

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C / **OC**: 414E / **Released**: eRA Commons User Name 07/17/2015
Award Processed: 06/15/2015 11:31:44 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI070908-07

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI070908-07

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget

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period.

e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI070908. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI070908-07

If you have a question on the award calculation or the terms and conditions of the award, your grants management contact should be **Qi Mai** at **(240) 669-5484** or via e-mail at **Qi.Mai@nih.gov.**

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an

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Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Raul A. Hernandez
Email: raul.hernandez@nih.gov **Phone**: 240-669-2957 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 240-627-3341 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI070908-07

INSTITUTION: KANSAS STATE UNIVERSITY

Budget	Year 7	Year 8	Year 9
Salaries and Wages	$139,958	$139,958	$139,958
Fringe Benefits	$40,858	$40,858	$40,858
Personnel Costs (Subtotal)	$180,816	$180,816	$180,816
Supplies	$87,425	$87,425	$87,425
Travel Costs	$9,801	$9,801	$9,801
Other Costs	$15,987	$15,987	$15,987
TOTAL FEDERAL DC	$294,029	$294,029	$294,029
TOTAL FEDERAL F&A	$147,015	$147,015	$147,015
TOTAL COST	$441,044	$441,044	$441,044

Facilities and Administrative Costs	Year 7	Year 8	Year 9
F&A Cost Rate 1	50%	50%	50%
F&A Cost Base 1	$294,029	$294,029	$294,029
F&A Costs 1	$147,015	$147,015	$147,015

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A. COVER PAGE

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression	
Grant Number: 5R01AI070908-07	**Project/Grant Period:** 08/01/2006 - 07/31/2018
Reporting Period: 08/01/2014 - 07/31/2015	**Requested Budget Period:** 08/01/2015 - 07/31/2016
Report Term Frequency: Annual	**Date Submitted:** 06/19/2015
Program Director/Principal Investigator Information: ROMAN R GANTA , PHD MS BS MOTH **Phone number:** 785-532-4612 **Email:** rganta@vet.k-state.edu	**Recipient Organization:** KANSAS STATE UNIVERSITY KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL, 1601 VATTIER STREET MANHATTAN, KS 665061103 **DUNS:** 929773554 **EIN:** 1480771751A1 **RECIPIENT ID:**
Change of Contact PD/PI: No	
Administrative Official: PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu	**Signing Official:** PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** Yes If yes, previously reported: No

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B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims: The central hypothesis of the project is that E. chaffeensis differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for pathogen's survival in vertebrate and tick cell environments. In support of this hypothesis, the project has specific aims; 1) Characterize E. chaffeensis RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression, 2) evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for E. chaffeensis in vivo growth, and 3) perform mutational analysis and in vivo screening to identify additional genes essential for the E. chaffeensis pathogenesis in vertebrate and tick hosts.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Gnta NIH R01 Progress report June 15 2015.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

We have several press releases regarding the research carried out as part of this funding and they were cited at numerous news papers and other means of news coverage. My expertise was also sought frequently by various citizens from the USA regarding the research we undertake and about the health issues they experience with tick-borne illnesses. Here are few examples of links where the news was published regarding our funding from the NIH.

http://www.k-state.edu/media/newsreleases/oct14/ganta10814.html?category=research&referredBy=K-State%20Today%20Home
http://veterinarynews.dvm360.com/k-state-researcher-looks-develop-vaccines-against-tick-borne-disease?rel=canonical
https://www.avma.org/News/JAVMANews/Pages/141201p.aspx

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Details of the planned research were included in the attachment file.

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A. SPECIFIC AIMS: The central hypothesis of the project is that *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for pathogen's survival in vertebrate and tick cell environments. In support of this hypothesis, the project has **specific aims**; 1) Characterize *E. chaffeensis* RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression, 2) evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth, and 3) perform mutational analysis and *in vivo* screening to identify additional genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts.

B. STUDIES AND RESULTS: **Specific Aim 1: Characterize *E. chaffeensis* RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression.** Considerable progress has been made in accomplishing the objectives of this aim. *E. chaffeensis* is an obligate intracellular tick-borne pathogen that replicates in both tick and vertebrate hosts. The ability of such pathogens to regulate gene expression in tick and vertebrate hosts is currently poorly understood. In prokaryotes, transcriptional specificity of promoters is controlled by sigma factors. *E. chaffeensis* genome contains genes for two sigma factors; sigma 32 (σ^{32}) and sigma70 (σ^{70}). It is difficult to study gene regulation in *E. chaffeensis* due to lack of transformation system. This is because this pathogen and related rickettsiales do not naturally harbor plasmids. We now developed an *E. coli*-based transcription system to study the *E. chaffeensis* transcriptional regulation. We used an *E. coli* strain where its endogenous σ^{70} gene expression is repressed with the *trp* repressible promoter and in its place *E. chaffeensis* recombinant σ^{70} expression is functionally substituted. This system and the previously established *in vitro* transcription system were used to map promoter motifs of two *E. chaffeensis* p28 Omp genes, as they are differentially expressed in tick and vertebrate hosts and their gene expression is driven primarily by the *E. chaffeensis* σ^{70}. We made numerous deletion and point mutation constructs spanning these two gene promoters to map the contributions of -10 and -35 motifs and the spacer sequences located between these two motifs. The study revealed that the highly conserved -35 motif of these two genes (TTGCTT) is very similar IN impacting the transcription, while -10 motifs do not contribute to promoter function. Further, we discovered that the high AT-rich spacer sequence located between the -10 and -35 motifs is distinct for the two differentially expressed genes and contributes to variations in the transcription. We further mapped the protein domains 4.2 and 1.1 of *E. chaffeensis* σ^{70} as interacting with the -35 motif and AT-rich spacer sequence, respectively. This work is the first in defining the molecular basis of differential gene expression by mapping promoter segments of two closely related and differentially expressed *E. chaffeensis* genes. These data are now compiled for Unpublished. The manuscript will be Unpublished

Specific Aim 2: Evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth. We assessed two clonally purified *E. chaffeensis* mutants with insertions within genes Ech_0379 and Ech_0660 as vaccine candidates in deer and dogs. Infection with Ech_0379 mutant and challenge with wild-type *E. chaffeensis* one month following inoculation with the mutant resulted in the reduced presence of the organism in blood compared to wild-type infection in both deer and dogs. The Ech_0660 mutant infection resulted in its rapid clearance from blood stream. The wild-type infection challenge following Ech_0660 mutant inoculation also caused the pathogen's rapid clearance from blood and tissue samples assessed at the end of the study. The Ech_0379 mutant infected and challenged animals remained positive for the organism in tissue samples in deer, but not in dogs. This is the first study that documents insertion mutations in *E. chaffeensis* that cause attenuated growth and confer protection against wild-type infection challenge. This study is important in developing immunogens to protect animals and people against Ehrlichia species infections. This portion of the research results is published recently in the journal, *Infection and Immunity* (2015).

We further studied the impact of mutations in *E. chaffeensis* on the transcription of genes near the insertion sites and demonstrated that they cause polar effects in altering gene expression at four genomic locations. We also reported mutations causing attenuated growth in deer (the pathogen's reservoir host) also caused attenuation in an incidental host (dog), but not in its tick vector, *Amblyomma americanum*. This is the first study documenting insertion mutations in *E. chaffeensis* cause polar effects in altering gene expression from the genes located upstream and downstream to insertion sites and differential requirements of

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functionally active genes of the pathogen for its persistence in vertebrate and tick hosts. This study is important in furthering our knowledge on *E. chaffeensis* pathogenesis. This portion of the research results is Unpublished

Specific Aim 3: Perform mutational analysis and *in vivo* screening to identify additional genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts. We created numerous constructs to improve the efficiency of random mutagenesis in *E. chaffeensis*. The modifications to the constructs include inserting the *Ehrlichia* origin of replication and highly active and constitutively expressing gene promoters. Further, we prepared several constructs in support of targeted mutagenesis. The goal here is to devise mutagenesis methods where the pathogen can tolerate insertions and gene disruptions, such as in the genomic regions where we already documented mutations previously. Further to promote rapid progress on this portion of the project, we established a sub-contract with our collaborator, Redacted by agreement Redacted by agreement This sub-contract will continue for 2016 budget period. We expect to make substantial project in this specific aim during next 12 months.

C. SIGNIFICANCE: We are the first and the only group in defining the molecular basis of differential gene expression by mapping promoter segments of two closely related and differentially expressed *E. chaffeensis* genes. In the absence of natural plasmids in *Ehrlichia* species and in the related *Anaplasmataceae* pathogens, transformations to study regulation of gene expression is extremely difficult. Our studies, therefore, are highly innovative in offering alternative ways to study how the pathogens regulate gene expression. Specifically, our establishment of surrogate *E. coli* system and *in vitro* transcription method to study regulation of gene expression will open ways to gain considerable understanding of the molecular basis for *Anaplasmataceae* pathogens' dual host adaptation. We are also the first to investigate the live attenuated mutants of *E. chaffeensis* for their value in conferring protection against wild-type infection challenge. These studies will lead to further studies to assess the potential of attenuated mutants as vaccines to protect animals and people against *E. chaffeensis* infection. The progress in generating additional mutants of the pathogen is critical for gaining insights about the pathogenesis of this important tick-borne pathogen causing the HME disease.

D. PLANS: We will continue our work on all three specific aims of the proposal to further the progress on the proposed goals. We will continue our studies in evaluating the promoter studies of genes regulated by sigma factors, σ^{32} and σ^{70}, of *E. chaffeensis* RNA polymerase by taking advantage of the *E. coli* surrogate and *in vitro* transcription methods which we developed as part of the progress made on this grant. We will also focus on studies to identify the pathogen regulatory proteins, which may contribute to altering gene expression by sensing host cell environments. We already made substantial progress in evaluating the significance of host-specific differential expression by characterizing mutations in two of the three genes identified as essential for *E. chaffeensis in vivo* growth. We will expand the studies to understand why the transcriptionally active Ech_0660 is necessary for the pathogen's persistent growth in vertebrate hosts. We are developing numerous antibodies to map gene expression from the genes located near the Ech_0660. We will use them to map protein expression and to define the impact of gene inactivation of Ech_0660. We will also work on developing targeted mutations in genes found to be critical for the pathogen's survival in vertebrate hosts. We expect to generate additional mutations within the genome of *E. chaffeensis*. The sub-contract with Redacted by is expected to facilitate rapid progress in identifying additional genes critical for the pathogenesis.

E. PUBLICATIONS (published or submitted):

1) Nair AD, Cheng C, Jaworski DC, Willard LH, Sanderson MW, Ganta RR. Ehrlichia chaffeensis infection in the reservoir host (white-tailed deer) and in an incidental host (dog) is impacted by its prior growth in macrophage and tick cell environments. PLoS One. 2014 Oct 10;9(10):e109056. doi: 10.1371/journal.pone.0109056. eCollection 2014. PubMed PMID: 25303515; PubMed Central PMCID: PMC4193820.

Obtained via FOIA by White Coat Waste Project

2) Raghavan RK, Neises D, Goodin DG, Andresen DA, Ganta RR. Bayesian spatio-temporal analysis and geospatial risk factors of human monocytic ehrlichiosis. PLoS One. 2014 Jul 3;9(7):e100850. doi: 10.1371/journal.pone.0100850. eCollection 2014. PubMed PMID: 24992684; PubMed Central PMCID: PMC4081574.

3) Stoffel RT, McClure JC, Butcher MM, Johnson GC, Roland W, Cheng C, Sirigireddy KR, Ganta R, Boughan K, Ewing SA, Stich RW. Experimental infection of Rhipicephalus sanguineus with Ehrlichia chaffeensis. Vet Microbiol. 2014 Aug 6;172(1-2):334-8. doi: 10.1016/j.vetmic.2014.04.017. Epub 2014 May 4. PubMed PMID: 24894131; PubMed Central PMCID: PMC4098862.

4) Paddock CD, Denison AM, Dryden MW, Noden BH, Lash RR, Abdelghani SS, Evans AE, Kelly AR, Hecht JA, Karpathy SE, Ganta RR, Little SE. High prevalence of "Candidatus Rickettsia andeanae" and apparent exclusion of Rickettsia parkeri in adult Amblyomma maculatum (Acari: Ixodidae) from Kansas and Oklahoma. Ticks Tick Borne Dis. 2015 Apr;6(3):297-302. doi: 10.1016/j.ttbdis.2015.02.001. Epub 2015 Mar 13. PubMed PMID: 25773931.

5) Nair AD, Cheng C, Jaworski DC, Ganta S, Sanderson MW, Ganta RR. Attenuated Mutants of Ehrlichia chaffeensis in Inducing Protection against Wild-type Infection Challenge in the Reservoir Host and in an Incidental Host. Infect Immun. 2015 Apr 27. pii: IAI.00487-15. [Epub ahead of print] PubMed PMID: 25916990.

Unpublished

Unpublished

F. ABSTRACTS:

1) Cheng C, Nair AD, Jaworski DC, Ganta S and Ganta RR. Attenuated transposon mutants of *Ehrlichia chaffeensis* induce protective host response against wild-type infection challenge. International conference: Global Summit on Emerging Science and Technologies: Impact on Environment and Human Health, August 1-3, 2014, Nellore, India

2) Raghavan RK, Goodin DA, Neises D, Anderson G, Ganta RR. Spatial Expansion of Tick-borne Human Monocytic Ehrlichiosis in the Midwestern United States. International Society of Geospatial Health, Oct 31st and Nov 2nd at New Orleans, LA

3) Kim DH, Nair ADS, C. Cheng C, Y. Park CY, Ganta RR and Jaworski DC. *Ehrlichia chaffeensis*-impacted transcriptome of Amblyomma americanum. May 12-17, 2015, Keystone Symposium on the Arthropod vector: the controller of transmission, Taos, NM

4) Ganta RR. *Ehrlichia chaffeensis* mutagenesis to identify genes critical for persistent infection and vaccine development. ESCCAR, International congress on *Rickettsia* and other intracellular bacteria. June 13-16, 2015, Auditoire César Roux University Hospital – CHUV, Lausanne, Switzerland

Obtained via FOIA by White Coat Waste Project

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

The attachment file included the details of publications generated from the project.

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

Obtained via FOIA by White Coat Waste Project

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	GANTA, ROMAN R.	PII		BS,MOTH,MS,PHD	PD/PI	EFFORT					NA
	N	Redacted by agreement			MS, PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N				MS, PhD	He was a post-doc on the grant, but he no longer works.						NA
eRA Commons User Name	Y				PHD,DVM	Co-Investigator						NA
	N				BS, MS	Microbiologist						NA
	N				BS	Technician						NA
eRA Commons User Name	N				PHD,BS, MS	Co-Investigator						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

Yes

The effort of Redacted by agreement is reduced to EFFORT and she is not listed as a key personnel. This change was made as we will now mostly use dogs in place of deer for experimental infection studies in support of specific aims 2 and 3. This change in experimental design was based on our recent published data which support that the infection assessment outcomes for deer and dog are very similar for E. chaffeensis infections and therefore we expect to accomplish project goals similarly as proposed. Redacted by is now included as a key person to the project as she is the PI for the sub-contract portion of the grant.

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

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D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

Obtained via FOIA by White Coat Waste Project

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

Obtained via FOIA by White Coat Waste Project

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

Obtained via FOIA by White Coat Waste Project

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Kansas State University	929773554	KS-001	2 Fairchild Hall Manhattan KS 665061103

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

Obtained via FOIA by White Coat Waste Project

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Obtained via FOIA by White Coat Waste Project

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/20/2016

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI070908-08
FAIN: R01AI070908

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Mr. Lowe, Paul R.
Asst Vice President/Research
2 Fairchild Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Period Of Performance:
Budget Period: 08/01/2016 – 07/31/2017
Project Period: 08/01/2006 – 07/31/2018

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $441,044 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI070908. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Obtained via FOIA by White Coat Waste Project

Laura A. Pone
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Obtained via FOIA by White Coat Waste Project

SECTION I – AWARD DATA – 5R01AI070908-08

Award Calculation (U.S. Dollars)

Salaries and Wages	$139,958
Fringe Benefits	$40,858
Personnel Costs (Subtotal)	$180,816
Materials & Supplies	$87,425
Travel	$9,801
Other	$15,987
Federal Direct Costs	$294,029
Federal F&A Costs	$147,015
Approved Budget	$441,044
Total Amount of Federal Funds Obligated (Federal Share)	$441,044
TOTAL FEDERAL AWARD AMOUNT	$441,044
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$441,044

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
8	$441,044	$441,044
9	$441,044	$441,044

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy, Immunology and Transplantation Research
CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908C
PMS Account Type:	P (Subaccount)
Fiscal Year:	2016

IC	CAN	2016	2017
AI	8472302	$441,044	$441,044

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C / **OC**: 414E / **Released:** eRA Commons User Name 07/19/2016
Award Processed: 07/20/2016 12:01:39 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI070908-08

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI070908-08

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.

Obtained via FOIA by White Coat Waste Project

e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI070908. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI070908-08

STAFF CONTACTS

Obtained via FOIA by White Coat Waste Project

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Azita Halary
Email: azita.halary@nih.gov **Phone**: 240-669-2991 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 240-627-3341 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI070908-08

INSTITUTION: KANSAS STATE UNIVERSITY

Budget	Year 8	Year 9
Salaries and Wages	$139,958	$139,958
Fringe Benefits	$40,858	$40,858
Personnel Costs (Subtotal)	$180,816	$180,816
Materials & Supplies	$87,425	$87,425
Travel	$9,801	$9,801
Other	$15,987	$15,987
TOTAL FEDERAL DC	$294,029	$294,029
TOTAL FEDERAL F&A	$147,015	$147,015
TOTAL COST	$441,044	$441,044

Facilities and Administrative Costs	Year 8	Year 9
F&A Cost Rate 1	50%	50%
F&A Cost Base 1	$294,029	$294,029
F&A Costs 1	$147,015	$147,015

Obtained via FOIA by White Coat Waste Project

A. COVER PAGE

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression	
Grant Number: 5R01AI070908-08	**Project/Grant Period:** 08/01/2006 - 07/31/2018
Reporting Period: 08/01/2015 - 07/31/2016	**Requested Budget Period:** 08/01/2016 - 07/31/2017
Report Term Frequency: Annual	**Date Submitted:** 06/16/2016
Program Director/Principal Investigator Information: ROMAN R GANTA , PHD MS BS **Phone number:** 785-532-4612 **Email:** rganta@vet.k-state.edu	**Recipient Organization:** KANSAS STATE UNIVERSITY KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL, 1601 VATTIER STREET MANHATTAN, KS 665061109 **DUNS:** 929773554 **EIN:** 1480771751A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu	**Signing Official:** PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

Obtained via FOIA by White Coat Waste Project

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims: The central hypothesis of the project is that E. chaffeensis differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for pathogen's survival in vertebrate and tick cell environments. In support of this hypothesis, the project has specific aims; 1) Characterize E. chaffeensis RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression, 2) evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for E. chaffeensis in vivo growth, and 3) perform mutational analysis and in vivo screening to identify additional genes essential for the E. chaffeensis pathogenesis in vertebrate and tick hosts.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Gnta NIH R01 Progress report June 2016.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Details of the planned research were included in the attachment file.

Obtained via FOIA by White Coat Waste Project

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

A. SPECIFIC AIMS: The central hypothesis of the project is that *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for pathogen's survival in vertebrate and tick cell environments. In support of this hypothesis, the project has **specific aims**: 1) Characterize *E. chaffeensis* RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression, 2) evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth, and 3) perform mutational analysis and *in vivo* screening to identify additional genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts.

B. STUDIES AND RESULTS: **Specific Aim 1: Characterize *E. chaffeensis* RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression.** We continue to make steady progress in accomplishing the objectives of this aim. *E. chaffeensis* is an obligate intracellular tick-borne pathogen that replicates in both tick and vertebrate hosts. Little is known about the pathogen gene regulation. Transcriptional specificity of genes in prokaryotes is accomplished by one or more of the sigma factors. *E. chaffeensis* contains only two sigma factors; sigma 32 (σ^{32}) and sigma70 (σ^{70}). As part of our prior progress of this grant application, we developed an *E. coli*-based transcription system to study the *E. chaffeensis* transcriptional regulation where we used an *E. coli* strain having its endogenous σ^{70} gene expression repressed with the *trp* repressible promoter and in its place *E. chaffeensis* recombinant σ^{70} expression functionally substituted. This system and the previously established *in vitro* transcription system (also accomplished as part of the prior progress on this grant) were used to map promoter motifs of two differentially expressed *E. chaffeensis* p28 Omp genes whose gene expression is primarily regulated by its σ^{70}. We made numerous deletion and point mutation constructs spanning p28-Omp 14 and 19 genes and mapped the -10 and -35 motifs and the AT rich spacers located between the two motifs by performing detailed mutational analysis. Mutations within the -35 motif of the genes impacted transcription differently, while -10 motif deletions had no impact. The AT-rich spacers also contributed to transcriptional differences. We further demonstrated that the domain 4.2 of *E. chaffeensis* σ^{70} is important for regulating promoter activity and the deletion of region 1.1 of *E. chaffeensis* σ^{70} causes enhancement of the promoter activity. In view of the lack of a transformation system in *E. chaffeensis* and in other related tick-borne intracellular rickettsial pathogens, the assessment of *Ehrlichia* transcriptional machinery in the surrogate *E. coli* system along with the validation experiments carried out by *in vitro* transcription assays offer innovative means in studying gene expression in *E. chaffeensis* and other important intracellular rickettsial pathogens belonging to the *Anaplasmataceae* family. These data are accepted Unpublished in press). Unpublished

Despite our significant progress, much remains to be understood about the regulation of gene expression. We initiated studies to test the hypothesis that one or more of the pathogen transcription regulators play an important role in regulating the gene expression. We initiated a targeted approach in characterizing five putative transcription regulators of *E. chaffeensis* (CtR, EcxR, HU, MerR and Tr1) for their roles in influencing the promoter activities of 7 genes encoding for ClpB, DnaK, GroES/L, HU, p28-Omp 14, p28-Omp 19, and Tr1. We recombinantly expressed the five transcription regulators and initiated the studies to assess their interactions with the promoter regions of the above listed 7 genes. Electrophoretic mobility shift assays (EMSAs) were performed with Tr1, CtR and EcxR and using the 7 promoter segments. Specific interactions were observed for all of the promoters with EcxR. Specific binding of the EcxR was independently verified by South-Western blot analysis for the promoters of ClpB, DnaK, GroeS/L, p28-Omp 14 and 19, and Tr1. We then investigated then impact of EcxR binding on the promoter activities of ClpB, DnaK, GroeS/L p28-Omp 14 and 19; the EcxR binding resulted in the variable levels of transcription enhancement from 20-60%. Finally, we confirmed specific binding of the EcxR to the Tr1, p28-Omp 14 and 19 gene promoters by Yeast one-hybrid assay. This is the first detailed study focused on evaluating the contributions of the putative transcription regulators of the pathogen and our studies demonstrate that the EcxR is a global regulator that binds and enhances the promoter activities of several *E. chaffeensis* genes. This area of research needs additional experimentation to further our understanding of the role of DNA binding proteins of E. chaffeensis in controlling transcriptional regulation. This work is presented at the recent American Society for Rickettsiology meeting (28th meeting of the ASR, Big Sky, MT, June 11-14, 2016; Abstract # 27; EcxR is a global gene regular in *Ehrlichia chaffeensis* by Jakkula et al.)

Specific Aim 2: Evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth. In our previous year's progress report, we reported the assessment of two clonally purified *E. chaffeensis* mutants with insertions within genes Ech_0379 and Ech_0660 as vaccine candidates in deer and dogs. Infection with Ech_0379 mutant offered notable reduction of systemic infection of wild-type challenged bacteria in both deer and dogs. We also reported that

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the Ech_0660 mutant offered complete clearance of the IV inoculation of the wild-type infection challenge from both blood and tissue samples assessed at the end of the study. The Ech_0379 mutant also offered complete clearance in the canine host when assessed in the tissue samples at the end point of the study in dogs, but not in deer. These data were already published last year (Nair et al. Infect. Immune. 2015). This year, we extended the vaccine study to investigate the immunological assessments in the canine host and also tested if the mutants confer protection against tick-transmission challenge. In particular, we demonstrated that vaccination with the Ech_0660 mutant protects dogs from physiological tick-transmitted challenge with wild-type pathogen. We also described for the first time the cellular and humoral immune responses induced by Ech_0660 mutant vaccination and wild-type *E. chaffeensis* infection challenge in the canine host. Both vaccination and infection induced a rise in E. chaffeensis-specific antibody titers and a significant Th1 response in peripheral blood as measured by E. chaffeensis antigen-dependent CD4+ T cell proliferation and IFNγ production. Further, we described for the first time significant IL-17 production by peripheral blood leukocytes from both Ech_0660 mutant vaccinated animals and wild-type infection control animals, suggesting a previously unrecognized role for IL-17 and Th17 cells in the immune response to rickettsial pathogens. Our results are a critical first step towards defining the role of the immune system in vaccine-induced protection from *E. chaffeensis* infection in an incidental host; and confirm the potential of the attenuated mutant clone, Ech_0660, to be used as a vaccine candidate for protection against tick-transmitted *E. chaffeensis* infection. These novel data were published this year in PLoS ONE (McGill et al. 2016).

As part of the work planned in this specific aim, we presented evidence that the mutations within the *E. chaffeensis* genome at four genomic locations cause polar effects in altering gene expressions. We also reported mutations causing attenuated growth in the pathogen's reservoir host and in an incidental host, but not in its tick vector, Amblyomma americanum. This study is important in furthering our knowledge on E. chaffeensis pathogenesis and the work was also published (Cheng et al. PLoS ONE, 2015). We have also developed a model for *E. chaffeensis* transmission that eliminates the entire tick acquisition stage while still producing high numbers of infected ticks that are also able to transmit infections to naïve hosts. Fully engorged *A. americanum* nymphs were ventrally needle-infected and following molting, the unfed adult ticks were used to infect naive deer and dogs. We have also described using the infected ticks generated by this method in transmitting both wild-type and transposon mutants of *E. chaffeensis* to its primary reservoir host (white tailed deer) and to another known incidental host (dog). The infection progression and IgG antibody responses in deer were similar to those observed with transmission feeding of ticks acquiring infection by natural blood feeding. The pathogen infections acquired by natural tick transmission and by feeding needle-infected ticks on animals were also similar to intravenous infections in causing persistent infections. The novel method of generating the needle-infected ticks having the ability to transmit pathogens will be a valuable resource to substantially simplify the process of generating infected ticks and to study infection systems in vertebrate hosts where interference of other pathogens could be avoided. This work is currently Unpublished

Unpublished

Specific Aim 3: Perform mutational analysis and *in vivo* screening to identify additional genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts. We created numerous constructs to improve the efficiency of random mutagenesis in *E. chaffeensis*. The modifications to the constructs include inserting the *Ehrlichia* origin of replication and highly active and constitutively expressing gene promoters. Further, we prepared several constructs in support of targeted mutagenesis. The goal here is to devise mutagenesis methods where the pathogen can tolerate insertions and gene disruptions, such as in the genomic regions where we already documented mutations previously. As part of this project period, we had a sub-contract with our collaborator, Dr. Ulrike Munderloh, the University of Minnesota. We performed mutational studies by both random Himar 1 mutagenesis and by targeted mutagenesis. We generated nearly 50 new mutants by random mutagenesis. We are currently characterizing these new mutants. Targeted mutagenesis of *Ehrlichia* species and in related rickettsiale pathogens remains a big challenge. Although we were successful and reported previously in creating targeted mutations in *E. chaffeensis,* stable mutations are documented at several genomic locations only by random mutagenesis approach. Further, our previous studies (microarray data; unpublished) revealed that over 94% of the predicted genes of the organism are transcribed when cultivated *in vitro* in vertebrate macrophages and tick cells. Based on these data, we hypothesized that it is a challenge to mutate a specific gene of interest in *E. chaffeensis*, as its genome has fewer redundant spots where targeted gene disruptions are possible. We then postulated that targeted mutagenesis is possible if we select a genomic region identified as mutatable by random mutagenesis. To test these hypotheses, we chose several regions to create targeted mutations, where we previously demonstrated insertion mutations by random mutagenesis. Plasmid constructs were prepared to include two fragments of DNA spanning the genes as 5' and 3' flanking sequences with a middle insertion containing the *aadA* gene to confer resistance to spectinomycin/streptomycin antibiotics. We engineered the *E. chaffeensis tuf* gene promoter to drive the expression of the *aadA* gene expression. The entire segments were then amplified from the plasmids as linear

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fragments and used to electroporate into cell-free *E. chaffeensis* organisms. The transformed bacteria were propagated in ISE6 tick cells in the presence of antibiotics to assess the homologous recombination. After two weeks, the antibiotic resistant organisms were observed. The presence of insertions in the *E. chaffeensis* genome was confirmed by PCR and sequence analysis targeting to the sequences flanking both the 5' and 3' to the insertion sites, and by Southern blot analysis using genomic DNA from the mutants. The targeted insertion mutants remained in culture stably for over two months and also grew well in the canine macrophage cell line, DH82. This is the first evidence demonstrating the generation of stable mutations by homologous recombination in *E. chaffeensis*. This work is also presented at this year's American Society for Rickettsiology meeting (28th meeting of the ASR, Big Sky, MT, June 11-14, 2016; Abstract # 71; Targeted mutagenesis in *Ehrlichia chaffeensis* by homologous recombination by Wang et al.)

C. SIGNIFICANCE: We developed several novel methods to promote the advancement of rickettsial research. In particular, we developed innovative methods in defining the molecular basis of differential gene expression by mapping promoters of several genes of *E. chaffeensis* genes and using the *E. coli* surrogate system, which we developed. We also extended studies in both random and targeted mutagenesis; this is also relatively new to *Ehrlichia* research. Targeted mutagenesis remains a major challenge for Anaplasmataceae field and we demonstrated for the first time that stable targeted mutations could be generated in *Ehrlichia*. These data are significant because studies can now be undertaken to investigate targeted mutations assess the functions of numerous genes and in several rickettsial pathogens. Our studies, therefore, continue to be highly innovative in offering new directions to study rickettsial molecular biology, biochemistry, cell biology and in defining pathogenesis. We are also the first in investigating the live attenuated vaccine against *E. chaffeensis* and these studies can also be extended to other related rickettsials.

D. PLANS: We will continue our work on all three specific aims of the proposal to further the progress on the proposed goals. We will continue our studies in evaluating the promoter studies of genes regulated by both σ^{32} and σ^{70} of *E. chaffeensis* RNA polymerase by taking advantage of the *E. coli* surrogate and *in vitro* transcription methods. We will also continue studies to further advance our knowledge in defining the contributions of the pathogen DNA binding proteins having gene regulatory function. We will continue the studies to understand functional significance of genes in which we inserted mutations. We will also map the genome sites of the newly developed random mutants. We expect to continue generating additional mutations within the genome of *E. chaffeensis* to facilitate continued progress in identifying additional genes critical for the pathogenesis.

E. PUBLICATIONS (published/in press):

1) Mutations in *Ehrlichia chaffeensis* causing polar effects in gene expression and differential host specificities. Cheng C, Nair ADS, Jaworski DC, and Ganta RR. PLoS ONE (2015 Jul 17;10(7):e0132657. doi: 10.1371/journal.pone.0132657. eCollection 2015. PMID: 26186429).

2) Maximum Entropy-Based Ecological Niche Model and Bio-Climatic Determinants of Lone Star Tick (Amblyomma americanum) Niche. Raghavan RK, Goodin DG, Hanzlicek GA, Zolnerowich G, Dryden MW, Anderson GA, Ganta RR. Vector Borne Zoonotic Dis. 2016 Jan 29. [Epub ahead of print] PMID: 26824880

3) Vaccination with an Attenuated Mutant of *Ehrlichia chaffeensis* Induces Pathogen-Specific CD4+ T Cell Immunity and Protection from Tick-Transmitted Wild-Type Challenge in the Canine Host. McGill JL, Nair AD, Cheng C, Rusk RA, Jaworski DC, Ganta RR. PLoS One. 2016 Feb 3;11(2):e0148229. doi: 10.1371/journal.pone.0148229. eCollection 2016. PMID: 26841025

4) Comparative Experimental Infection Study in Dogs with *Ehrlichia canis, E. chaffeensis, Anaplasma platys* and *A. phagocytophilum*. Nair AD, Cheng C, Ganta CK, Sanderson MW, Alleman AR, Munderloh UG, Ganta RR. PLoS One. 2016 Feb 3;11(2):e0148239. doi: 10.1371/journal.pone.0148239. eCollection 2016. PMID: 26840398

5) Hierarchical Bayesian Spatio–temporal Analysis of Climatic and Socio–economic Determinants of Rocky Mountain Spotted Fever. Raghavan RK, Goodin DG, Neises D, Anderson GA, and Ganta RR. PLoS ONE, 2016; 4;11(3):e0150180. doi: 10.1371/journal.pone.0150180. eCollection 2016. PMID: 26942604

6) Bayesian Space-Time Patterns and Climatic Determinants of Bovine Anaplasmosis. Hanzlicek GA, Raghavan RK, Ganta RR, Anderson GA. PLoS One. 2016 Mar 22;11(3):e0151924. doi: 10.1371/journal.pone.0151924. eCollection 2016. PMID: 27003596

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7) Unpublished

F. ABSTRACTS:

Presented at the 28th Meeting of the American Society for Rickettsiology, June 11-14, 2016, at the Big Sky Resort in Big Sky, Montana.

1) Abstract # 27: EcxR is a global gene regular in Ehrlichia chaffeensis. Laxmi U. M. Jakkula, Huitao Liu, Ying Wang and Roman Ganta.

2) Abstract # 69: Metabolic and biosynthetic activity of Ehrlichia chaffeensis in a host cell-free medium Vijay K. Eedunuri1, Chuanmin Cheng, Anders Omsland, Dan Boyle, and Roman R. Ganta.

3) Abstract # 71: Targeted mutagenesis in Ehrlichia chaffeensis by homologous recombination. Ying Wang, Chuanmin Cheng and Roman R. Ganta.

4) Abstract # 72: Monovalent Cation/Sodium:Proton Antiporter Proteins of Ehrlichia chaffeensis. Lanjing Wei, Huitao Liu, and Roman R. Ganta.

5) Abstract # 82: A candidate vaccine induces cellular and humoral immunity and protects from tick-transmitted Ehrlichia chaffeensis. Jodi McGill, Arathy Nair, Chuanmin Cheng, Rachel Rusk, Deborah Jaworski and Roman Ganta

6) Abstract # 90: Consistencies in spatio-temporal trends, and climate and other geospatial risk factors for rickettsial diseases in the central Midwestern states. Ram K Raghavan, Gary Anderson, Doug Goodin, and Roman Ganta

7) Abstract # 136: Protein aggregation in Ehrlichia chaffeensis during macrophage infection. Dorota Kuczynska-Wisnik, Michal Zolkiewski, and Roman R. Ganta.

Presented at the 27th Meeting of the American Society for Rickettsiology, June 20-23, 2015 Village at Squaw Valley, Olympic Valley, California

8) Abstract # 8) Abstract #113: Needle-inoculated ticks are competent to transmit Ehrlichia chaffeensis to naïve hosts D.C. Jaworski, C. Cheng, A.S. Nair, and R.R. Ganta

9) Abstract #43: Sequence Determinants Spanning -35 motif and Spacer Sequences Impacting Ehrlichia chaffeensis Sigma 70-Dependent Promoter Activity of Two p28 Outer Membrane Protein Genes. Huitao Liu Laxmi UMR Jakkula , Tonia Von-Ohlen and Roman R. Ganta

10) Abstract #77: Attenuated Mutants of Ehrlichia chaffeensis in Inducing Protection against Wild-type InfectioN Challenge in the Reservoir Host and in an Incidental Host. Chuanmin Cheng1# , Arathy D. S. Nair1#, Deborah C. Jaworski1,2, Suhasini Ganta3 and Roman R. Ganta

11) Abstract #87: The molecular chaperone ClpB supports the infectious stage of Ehrlichia chaffeensis Ting Zhang, Huitao Liu, Chuanmin Cheng, Arathy Nair, Roman Ganta, and Michal Zolkiewski

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C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Paddock CD, Denison AM, Dryden MW, Noden BH, Lash RR, Abdelghani SS, Evans AE, Kelly AR, Hecht JA, Karpathy SE, Ganta RR, Little SE. High prevalence of "Candidatus Rickettsia andeanae" and apparent exclusion of Rickettsia parkeri in adult Amblyomma maculatum (Acari: Ixodidae) from Kansas and Oklahoma. Ticks Tick Borne Dis. 2015 Apr;6(3):297-302. PubMed PMID: 25773931; PubMed Central PMCID: PMC4487539.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

The attachment file included the details of publications generated from the project.

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

Obtained via FOIA by White Coat Waste Project

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	GANTA, ROMAN R.	PII		BS,MS,PHD	PD/PI	EFFORT					NA
	N	Redacted by agreement			BVSc, MVSc, PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N				PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N				PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N				PHD,BS, MS	Co-Investigator						NA
	N				MS	Graduate Student (research assistant)						NA
	N				PHD,DVM	Co-Investigator						NA
	N				BS, MS	Technician						NA
	N				MS, PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

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Yes

Redacted by was included as a key person to the project last year as she is the PI for the sub-contract portion of the grant. We will not be having the subcontract this year for her.

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

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E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

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F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

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G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Kansas State University	929773554	KS-001	2 Fairchild Hall Manhattan KS 665061103
KANSAS STATE UNIVERSITY	929773554		KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL, 1601 VATTIER STREET MANHATTAN KS 665061109
Kansas State University	929773554	KS-001	2 Fairchild Hall Manhattan KS 665061103

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G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

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Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 06/28/2019

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI070908-09 REVISED
FAIN: R01AI070908

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression

Mr. Lowe, Paul R.
Asst Vice President/Research
2 Fairchild Hall
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Period Of Performance:
Budget Period: 08/01/2017 – 07/09/2019
Project Period: 12/15/2007 – 07/09/2019

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI070908. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Obtained via FOIA by White Coat Waste Project

Gregory P. Smith
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

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SECTION I – AWARD DATA – 5R01AI070908-09 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$294,029
Federal F&A Costs	$147,015
Approved Budget	$441,044
Total Amount of Federal Funds Obligated (Federal Share)	$441,044
TOTAL FEDERAL AWARD AMOUNT	$441,044

AMOUNT OF THIS ACTION (FEDERAL SHARE) $0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
9	$441,044	$441,044

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908C
PMS Account Type:	P (Subaccount)
Fiscal Year:	2017

IC	CAN	2017
AI	8472302	$441,044

NIH Administrative Data:
PCC: M46C / **OC**: 414E / **Released:** eRA Commons User Name 06/27/2019
Award Processed: 06/28/2019 12:02:18 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI070908-09 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI070908-09 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V,

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Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI070908. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) quarterly cash transaction data. A final quarterly federal cash transaction report is not required for awards in PMS B subaccounts (i.e., awards to foreign entities and to Federal agencies). NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures or quarterly federal cash transaction reporting. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last recorded cash drawdown level. If the grantee submits a final expenditure FFR but does not reconcile any discrepancies between expenditures reported on the final expenditure FFR and the last cash report to PMS, NIH will close the award at the lower amount. This could be considered a debt or result in disallowed costs.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm. This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, D42, D43, D71, DP7, G07, G08, G11, K12, K16, K30, P09, P40, P41, P51, R13, R25, R28, R30, R90, RL5, RL9, S10, S14, S15, U13, U14, U41, U42, U45, UC6, UC7, UR2, X01, X02.

Unless an application for competitive renewal is submitted, a Final Research Performance Progress Report (Final RPPR) must also be submitted within 120 days of the period of

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performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the Interim or Final RPPR. *Note that data reported within Section I of the Interim and Final RPPR forms will be made public and should be written for a lay person audience*.

NIH strongly encourages electronic submission of the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final invention statement may be e-mailed as PDF attachments to: NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final invention statement may be faxed to the NIH Division of Central Grants Processing, Grants Closeout Center, at 301-480-2304, or mailed to:

National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Suite 5016, MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express deliveries only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final RPPR is not required. However, a final expenditure FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI070908-09 REVISED

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

REVISED AWARD: This award is revised to adjust the budget and project period end dates due to the funding of 2 **R01** AI **070908-10** effective **07/10/2019**.

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Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as

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well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Michael W. Fato
Email: mfato@niaid.nih.gov **Phone**: 240-669-2968 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 240-627-3341 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI070908-09 REVISED

INSTITUTION: KANSAS STATE UNIVERSITY

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Facilities and Administrative Costs	Year 9
F&A Cost Rate 1	50%
F&A Cost Base 1	$294,029
F&A Costs 1	$147,015

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A. COVER PAGE

Project Title: Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression	
Grant Number: 5R01AI070908-09	**Project/Grant Period:** 08/01/2006 - 07/31/2018
Reporting Period: 08/01/2016 - 07/31/2017	**Requested Budget Period:** 08/01/2017 - 07/31/2018
Report Term Frequency: Annual	**Date Submitted:** 06/12/2017
Program Director/Principal Investigator Information: ROMAN R GANTA , BS MS PHD **Phone number:** 785-532-4612 **Email:** rganta@vet.k-state.edu	**Recipient Organization:** KANSAS STATE UNIVERSITY KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL, 1601 VATTIER STREET MANHATTAN, KS 665061109 **DUNS:** 929773554 **EIN:** 1480771751A1 **RECIPIENT ID:**
Change of Contact PD/PI: No	
Administrative Official: PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu	**Signing Official:** PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

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B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims: The central hypothesis of the project is that E. chaffeensis differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for pathogen's survival in vertebrate and tick cell environments. In support of this hypothesis, the project has specific aims; 1) Characterize E. chaffeensis RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression, 2) evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for E. chaffeensis in vivo growth, and 3) perform mutational analysis and in vivo screening to identify additional genes essential for the E. chaffeensis pathogenesis in vertebrate and tick hosts.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Gnta NIH R01 Progress report June 2 2017.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We will continue our work on all three specific aims of the proposal to further the progress on the proposed goals. We will continue our studies in evaluating the promoter studies of genes regulated by both σ32 and σ70 of E. chaffeensis RNA polymerase by taking advantage of the novel methods we developed using the E. coli surrogate and in vitro transcription methods. We will also continue our efforts in defining the contributions of the pathogen DNA binding proteins having gene regulatory function. We will also continue the research to understand functional significance of genes by taking advantage of both the targeted and random mutagenesis methods.

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A. SPECIFIC AIMS: The project goals are to study how *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals for its survival in vertebrate and tick cell environments. Three **specific aims** of the project are 1) characterize *E. chaffeensis* RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression, 2) evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth, and 3) perform mutational analysis and *in vivo* screening to identify additional genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts.

B. STUDIES AND RESULTS: **Specific Aim 1: Characterize *E. chaffeensis* RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression.** This is a very complex and challenging goal, like in other obligate bacteria, most of the RNA polymerase characterization must be performed in vitro and requires novel tools. Substantial progress has been made in this aim; *E. chaffeensis* is an obligate intracellular tick-borne pathogen that replicates in both tick and vertebrate hosts. As transcriptional specificity of genes in prokaryotes is accomplished by the RNA polymerase binding proteins known as sigma factors. *E. chaffeensis* has only two sigma factors; sigma 32 (σ^{32}) and sigma70 (σ^{70}). Our prior studies led to the development of novel in vivo transcription system where we took advantage of our newly developed surrogate system in *E. coli* to study *E. chaffeensis* transcriptional regulation. In particular, we used an *E. coli* strain where its own σ^{70} gene expression is repressed with the *trp* repressible promoter and is complemented with *E. chaffeensis* recombinant σ^{70} expression. This system, together with the *in vitro* transcription system developed as part of our funded project was used to map promoter motifs of two differentially expressed *E. chaffeensis* p28 Omp genes whose gene expression is primarily regulated by its σ^{70}. We are the first and the only group to define the detailed mapping of promoter segments of the two differentially expressed outer membrane protein genes; p28-Omp 14 and 19. This work was published late last year in a high impact peer review scientific journal, DNA Research (Liu *et al.* 2016). The assessment of *Ehrlichia* transcriptional machinery in the surrogate *E. coli* system along with the validation experiments carried out by *in vitro* transcription assays opened novel ways of studying gene expression in *E. chaffeensis* and other important intracellular rickettsial pathogens belonging to the *Anaplasmataceae* family. We now extended similar studies to another important gene likely involved in regulating *E. chaffeensis* stress, a chaperone protein, DnaK. We completed the promoter analysis to define the sequence determinants spanning -10 motif and spacer region of the DnaK that is regulated by the RNA polymerase binding sigma factor, Sigma 32. We developed an *E. coli*-based transcription system to study *E. chaffeensis* transcriptional regulation. An *E. coli* strain (CAG57101), where its chromosomal σ^{32} is inactivated, was used to express *E. chaffeensis* σ^{32} protein from a plasmid under the control of IPTG inducible P_{lac} *promoter.* The *E. coli* system and *in vitro* transcription system were used to map the -10 motif and the spacers located between the -10 and -35 motifs of *DnaK* promoter by performing detailed mutational analysis. We also further studied the function of domain1.0, 2.4 and 3.0 of *E. chaffeensis* σ^{32} for regulating promoter activity. This is the first study defining the -10 motif of Sigma 32-dependent promoter. His work is in its final stages to be compiled as a manuscript for publication in a Unpublished in preparation).

We previously reported that a molecular chaperone ClpB is upregulated in *E. chaffeensis* during its replication inside infected mammalian cells (Zhang et al. 2013). ClpB belongs to the AAA+ family of ATPases associated with different cellular activities, a group of 62 energy-dependent conformation-remodeling factors. We now investigated the extent of protein aggregation in *E. chaffeensis* during infection of canine macrophage cell line, DH82. We discovered that the size of the aggregated fraction of *E. chaffeensis* proteins increased during the first 48 h post infection. We also incubated the infected cells with guanidinium chloride (GuHCl), a known inhibitor of the protein-disaggregating molecular chaperone ClpB. Up to 0.5 mM GuHCl had no impact on the host cells, whereas the viability of the pathogen was reduced by ~60% in the presence of the inhibitor. Furthermore, we found that the size of the aggregated protein fraction in *E. chaffeensis* increased significantly in cultures supplemented with 0.5 mM GuHCl, which also resulted in the preferential accumulation of ClpB with the aggregated proteins. Together, the data suggest that an exposure of *E. chaffeensis* to the stressful environment of a host cell results in an increased aggregation of the pathogen's proteins, which is exacerbated upon inhibition of ClpB. Our studies for the first time established a link between protein quality control and pathogen survival during infection of a host. These data were published recently (Kuczynska-Wisnik *et al*. 2017).

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As summarized in the last year's progress report, we initiated studies to test the hypothesis that one or more of the pathogen transcription regulators play an important role in regulating the gene expression. Our targeted approach involved in characterizing five putative transcription regulators of *E. chaffeensis* (CtrA, EcxR, HU, MerR and Tr1) for their roles in influencing the promoter activities of 7 genes encoding for ClpB, DnaK, GroES/L, HU, p28-Omp 14, p28-Omp 19, and Tr1. We have prepared these proteins by using an *E. coli* expression system and used them to assess their interactions with the promoter regions of the above listed 7 genes. Specific interactions of the promoters with one of the five proteins, EcxR, were observed and that the interactions were verified by several independent methods which included Electrophoretic Mobility Shift Assay (EMSA),South-Western blot analysis and Yeast hybrid system. This is the first detailed study focused on evaluating the contributions of the putative transcription regulators of the pathogen and our studies demonstrate for the first time that EcxR is a global regulator in *E. chaffeensis* that binds to promoter regions of several *E. chaffeensis* genes and also alter the gene expression. This work is also in its final stages of compiling the data for publication in a Unpublished in preparation.)

Specific Aim 2: Evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth. Earlier, we reported the assessment of two clonally purified *E. chaffeensis* mutants with insertions within genes Ech_0379 and Ech_0660 as vaccine candidates in deer and dogs. Infection with Ech_0379 mutant offered moderate protection as evidenced by reduction of circulating bacteria of wild type challenged bacteria in both deer and dogs. Whereas, the Ech_0660 mutant offered complete protection as evidenced by complete clearance of the organism following wild type infection challenge from blood and tissue samples. Immunological assessments were also carried out to define differences in the host responses following vaccinations with Ech_0379 or Ech_0660 mutant and after challenges with wild type infections in canine infection model. All in all, we are the first to demonstrate the utility of attenuated vaccines to protect against *E. chaffeensis* infection. In particular, our studies not only are important in defining the role of host immune system in vaccine-induced protection from *E. chaffeensis* infection in an incidental host, but also open the way for expanding the continued progress towards vaccine development.

During the current progress year of the project, we developed targeted mutant organisms of *E. chaffeensis* with mutations in Ech_0230 and Ech_0379 genes (provided more details below). As our prior studies with several random mutants of *E. chaffeensis* in deer and dogs suggested that Ech_0230 gene mutant organisms clear rapidly from the hosts following experimental infection, we conducted studies to determine the potential of this mutant in conferring protection from wild type *E. chaffeensis* infection. In particular, we tested our recently developed targeted Ech_0230 gene mutant clone of *E. chaffeensis* for its value in offering protection against wild type pathogen infection challenge using canine host model. Consistent with our prior observations, the targeted Ech_0230 gene mutant organisms cleared rapidly from the canine host (tested in five animals). All five animals developed *E. chaffeensis*-specific antibody response to detectible levels starting from day 7 post vaccination, while non-vaccinated dogs had no antibody response. However, the Ech_0230 mutant organism vaccinated dogs were not protected from wild type *E. chaffeensis* challenge. This observation differed from our prior report for vaccination with Ech_0379 or Ech_0660 mutant organisms. These data demonstrate that host responses vary for different attenuated mutants of *E. chaffeensis.* Contrary to the variations in conferring protection, we observed a rise in the pathogen-specific antibody response following vaccination with Ech_0230 mutant organisms that is similar to non-vaccinated dogs receiving wild type pathogen infection challenge. However, *E. chaffeensis*-specific $CD4^+$ T-cell proliferation and cytokine responses were not detectable following vaccination with the Ech_0230 mutant organism, and the cellular response was similar in both vaccinated and non-vaccinated animals following wild type infection challenges. For the first time, we also demonstrated the expansion of a $CD4^+CD8^+$ double positive T-cell population in dogs infected with *E. chaffeensis*, and determined that this population participates in immunity to *E. chaffeensis* infection through antigen-specific secretion of proinflammatory cytokines and cytotoxic factors. We are currently compiling the Ech_0230 mutant vaccine study results for publication in a Unpublished Unpublished in preparation).

In last year's progress report, we described the novel method of generating needle-infected ticks having the ability to transmit *E. chaffeensis* to vertebrate hosts. This will be a valuable resource to substantially simplify the process of generating infected ticks and to study infection systems in vertebrate hosts where interference of other pathogens could be avoided. This work is now published in a peer reviewed journal (Jaworski et al. 2017).

Specific Aim 3: Perform mutational analysis and *in vivo* screening to identify additional genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts. The primary goal of this aim is to improve mutagenesis methods where the pathogen can tolerate insertion mutations with or without gene disruptions. Our random mutagenesis studies led to the development of over 50 new mutations in *E. chaffeensis* genome. This year,

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we competed the mapping of all these mutations to identify insertion sites. Several novel mutations are identified which included two p28 major outer membrane protein genes encoding for p28-1 and Omp-1V (Ech_1144 and Ech_1127, respectively), 120 kDa immunodominant surface protein gene (Ech_0039), a signal recognition particle protein gene (Ech_0475), RNA polymerase sigma factors sigma-32 and -70 genes (Ech_0655 and Ech_0760, respectively), four predicted type IV secretion systems (T4SS) effector protein genes (Ech_0187, Ech_1127, Ech_1038 and Ech_0945). We are now expanding the investigations to define the impact of the mutations on the *E. chaffeensis* grown *in vitro*, gene expression changes assessed by RNA and protein analysis as well as by monitoring the possible bacterial growth kinetics changes Unpublished in progress).

Targeted mutagenesis of *Ehrlichia* species and in other rickettsial pathogens belonging to the genera *Anaplasma* and *Rickettsia* remained continual challenge. Earlier, we reported targeted mutations in *E. chaffeensis,* however, stable targeted mutation did not survive for longer periods of time. We focused our efforts in developing mutagenesis methods, which work well in creating targeted mutations to both disrupt and restore a gene function. Lack of an efficient system for targeted mutagenesis in obligates remains a major impediment in understanding microbial pathogenesis. Challenges in creating targeted mutations may be attributed to essential nature of majority of the genes and intracellular replication dependence. Despite success in generating several random mutations by transposon mutagenesis by our group, presently a method that works well in creating mutations in specific genes of interest followed by complementation remains problematic for obligates and is also a highly sought-after goal. We now developed protocols to generate stable targeted mutations by allelic exchange method in *E. chaffeensis,* an obligate intracellular tick-borne bacterium responsible for the disease, human monocytic ehrlichiosis. Targeted mutations in *E. chaffeensis* were created to disrupt two genes (Ech_0230 and Ech_0379), and also to restore one gene (Ech_0379) by another allelic exchange mutation leading to the restoration of transcription from the inactivated gene from its own promoter and the recovered organisms also express mCherry which distinguishes from the wild type. This work is compiled as a manuscript that is currently under review at the Unpublished

C. SIGNIFICANCE: We continue to develop several novel methods to promote the advancement of rickettsial research. In particular, we established innovative methods in defining the molecular basis of differential gene expression by mapping promoters of several genes of *E. chaffeensis* genes. We also extended studies in both random and targeted mutagenesis; this is also relatively new to *Ehrlichia* research. Targeted mutagenesis remains a major challenge for Anaplasmataceae field and we the first to demonstrate the development of methods to create stable targeted mutations to both disrupt and restore a gene in *E. chaffeensis*. These studies are significant because research can now be undertaken by taking advantage of mutagenesis methods developed by us for investigating defining the functions of many hypothetical genes of several rickettsial pathogens. Our studies, therefore, continue to be highly innovative in offering new directions to study rickettsial molecular biology, biochemistry, cell biology and in defining pathogenesis. We are also the first in investigating the live attenuated vaccine against *E. chaffeensis* and these studies can also be extended to other related rickettsials.

D. PLANS: We will continue our work on all three specific aims of the proposal to further the progress on the proposed goals. We will continue our studies in evaluating the promoter studies of genes regulated by both σ^{32} and σ^{70} of *E. chaffeensis* RNA polymerase by taking advantage of the novel methods we developed using the *E. coli* surrogate and *in vitro* transcription methods. We will also continue our efforts in defining the contributions of the pathogen DNA binding proteins having gene regulatory function. We will also continue the research to understand functional significance of genes by taking advantage of both the targeted and random mutagenesis methods.

E. PUBLICATIONS:

1) Liu H, Jakkula LU, Von Ohlen T, Ganta RR. Sequence determinants spanning -35 motif and AT-rich spacer region impacting Ehrlichia chaffeensis Sigma 70-dependent promoter activity of two differentially expressed p28 outer membrane protein genes. ***DNA Res.*** 2016 Jul 8. pii: dsw034. [Epub ahead of print] PMID: 27402867

2) Raghavan RK, Goodin DG, Dryden MW, Hroobi A, Gordon DM, Cheng C, Nair AD, Jakkula LU, Hanzlicek GA, Anderson GA, Ganta RR. Heterogeneous Associations of Ecological Attributes with Tick-Borne Rickettsial Pathogens in a Periurban Landscape. ***Vector Borne Zoonotic Dis.*** 2016 Sep;16(9):569-76. doi: 10.1089/vbz.2016.1975. Epub 2016 Jul 25. PMID: 27454144

3) Jaworski DC, Cheng C, Nair AD, Ganta RR. Amblyomma americanum ticks infected with in vitro cultured wild type and mutants of Ehrlichia chaffeensis are competent to produce infection in naïve deer and dogs. ***Ticks Tick Borne Dis.*** 2017 Jan;8(1):60-64. doi: 10.1016/j.ttbdis.2016.09.017. Epub 2016 Sep 28. PMID:

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4) Wilkerson MJ, Black KE, Lanza-Perea M, Sharma B, Gibson K, Stone DM, George A, Nair AD, Ganta RR. Initial development and preliminary evaluation of a multiplex bead assay to detect antibodies to *Ehrlichia canis, Anaplasma platys,* and *Ehrlichia chaffeensis* outer membrane peptides in naturally infected dogs from Grenada, West Indies. ***J Vet Diagn Invest.*** 2017 Jan;29(1):109-114. doi: 10.1177/1040638716671979. Epub 2016 Nov 16. PMID: 27852813

5) Kuczynska-Wisnik D, Cheng C, Ganta RR, Zolkiewski M. Protein aggregation in Ehrlichia chaffeensis during infection of mammalian cells. ***FEMS Microbiol Lett.*** 2017 Mar 1;364(6). doi: 10.1093/femsle/fnx059. PMID: 28333306

Book Chapters:

1) Book title: Rickettsiales: Biology, Epidemiology, Molecular Biology and Vaccine Development, Ed. Sunil Thomas (Ed.), Springer Publishing (ISBN 978-3-319-46859-4), December, 2016.
Book chapter title: Mutagenesis in Ehrlichia and Anaplasma species: it's application for studies focused on understanding the pathogenesis and vaccine development. Roman R. Ganta

2) Book title: Rickettsiales: Biology, Epidemiology, Molecular Biology and Vaccine Development, Ed. Sunil Thomas (Ed.), Springer Publishing (ISBN 978-3-319-46859-4), December, 2016.
Book chapter title: Identification of T cell epitopes in the murine host response to Ehrlichia chaffeensis. Stephen K. Chapes, M. Teresa Ortega, Chuanmin Cheng, and Roman R. Ganta

F. ABSTRACTS:

McGill JL, Y Wang, G Boorgula, R R Ganta. 2017. Canine CD4+CD8+ double-positive T cells participate in immunity to Ehrlichia chaffeensis infection. American Association of Immunologists Annual Conference, Washington DC

G. PATENTS:

Ganta, R. R., Cheng, C. M, Nair, A, Jaworski, D, Ganta, S. Attenuated vaccines to protect against tick-borne Ehrlichia species infections. Patent Number: WO 2016118512 A1 Issue date: Jul 28, 2016.

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C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Cheng C, Nair AD, Indukuri VV, Gong S, Felsheim RF, Jaworski D, Munderloh UG, Ganta RR. Targeted and random mutagenesis of Ehrlichia chaffeensis for the identification of genes required for in vivo infection. PLoS pathogens. ;9(2):e1003171. PubMed PMID: 23459099; PubMed Central PMCID: PMC3573109.
Complete	Cheng C, Nair AD, Jaworski DC, Ganta RR. Mutations in Ehrlichia chaffeensis Causing Polar Effects in Gene Expression and Differential Host Specificities. PloS one. ;10(7):e0132657. PubMed PMID: 26186429; PubMed Central PMCID: PMC4505860.
Complete	Dedonder SE, Cheng C, Willard LH, Boyle DL, Ganta RR. Transmission electron microscopy reveals distinct macrophage- and tick cell-specific morphological stages of Ehrlichia chaffeensis. PloS one. ;7(5):e36749. PubMed PMID: 22615806; PubMed Central PMCID: PMC3352939.
Complete	Faburay B, Liu H, Peddireddi L, Ganta RR. Isolation and characterization of Ehrlichia chaffeensis RNA polymerase and its use in evaluating p28 outer membrane protein gene promoters. BMC microbiology. ;11:83. PubMed PMID: 21513529; PubMed Central PMCID: PMC3108270.
Complete	Jaworski DC, Cheng C, Nair AD, Ganta RR. Amblyomma americanum ticks infected with in vitro cultured wild-type and mutants of Ehrlichia chaffeensis are competent to produce infection in naïve deer and dogs. Ticks and tick-borne diseases. ;8(1):60-64. PubMed PMID: 27729288; PubMed Central PMCID: PMC5268845.
Complete	Kuczynska-Wisnik D, Cheng C, Ganta RR, Zolkiewski M. Protein aggregation in Ehrlichia chaffeensis during infection of mammalian cells. FEMS microbiology letters. ;364(6). PubMed PMID: 28333306; PubMed Central PMCID: PMC5399918.
Complete	Liu H, Von Ohlen T, Cheng C, Faburay B, Ganta RR. Transcription of Ehrlichia chaffeensis genes is accomplished by RNA polymerase holoenzyme containing either sigma 32 or sigma 70. PloS one. ;8(11):e81780. PubMed PMID: 24278458; PubMed Central PMCID: PMC3836757.
Complete	Liu H, Jakkula LU, Von Ohlen T, Ganta RR. Sequence determinants spanning -35 motif and AT-rich spacer region impacting Ehrlichia chaffeensis Sigma 70-dependent promoter activity of two differentially expressed p28 outer membrane protein genes. DNA research : an international journal for rapid publication of reports on genes and genomes. PubMed PMID: 27402867; PubMed Central PMCID: PMC5066175.
Complete	McGill JL, Nair AD, Cheng C, Rusk RA, Jaworski DC, Ganta RR. Vaccination with an Attenuated Mutant of Ehrlichia chaffeensis Induces Pathogen-Specific CD4+ T Cell Immunity and Protection from Tick-Transmitted Wild-Type Challenge in the Canine Host. PloS one. ;11(2):e0148229. PubMed PMID: 26841025; PubMed Central PMCID: PMC4739596.
Complete	Nair AD, Cheng C, Jaworski DC, Ganta S, Sanderson MW, Ganta RR. Attenuated Mutants of Ehrlichia chaffeensis Induce Protection against Wild-Type Infection Challenge in the Reservoir Host and in an Incidental Host. Infection and immunity. ;83(7):2827-35. PubMed PMID: 25916990; PubMed Central PMCID: PMC4468539.
Complete	Nair AD, Cheng C, Jaworski DC, Willard LH, Sanderson MW, Ganta RR. Ehrlichia chaffeensis infection in the reservoir host (white-tailed deer) and in an incidental host (dog) is impacted by its prior growth in macrophage and tick cell environments. PloS one. ;9(10):e109056. PubMed PMID: 25303515; PubMed Central PMCID: PMC4193820.
Complete	Nair AD, Cheng C, Ganta CK, Sanderson MW, Alleman AR, Munderloh UG, Ganta RR.

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	Comparative Experimental Infection Study in Dogs with Ehrlichia canis, E. chaffeensis, Anaplasma platys and A. phagocytophilum. PloS one. ;11(2):e0148239. PubMed PMID: 26840398; PubMed Central PMCID: PMC4739612.
Complete	Raghavan RK, Neises D, Goodin DG, Andresen DA, Ganta RR. Bayesian spatio-temporal analysis and geospatial risk factors of human monocytic ehrlichiosis. PloS one. ;9(7):e100850. PubMed PMID: 24992684; PubMed Central PMCID: PMC4081574.
Complete	Raghavan RK, Goodin DG, Dryden MW, Hroobi A, Gordon DM, Cheng C, Nair AD, Jakkula LU, Hanzlicek GA, Anderson GA, Ganta RR. Heterogeneous Associations of Ecological Attributes with Tick-Borne Rickettsial Pathogens in a Periurban Landscape. Vector borne and zoonotic diseases (Larchmont, N.Y.). ;16(9):569-76. PubMed PMID: 27454144; PubMed Central PMCID: PMC5011634.
Complete	Raghavan RK, Goodin DG, Hanzlicek GA, Zolnerowich G, Dryden MW, Anderson GA, Ganta RR. Maximum Entropy-Based Ecological Niche Model and Bio-Climatic Determinants of Lone Star Tick (Amblyomma americanum) Niche. Vector borne and zoonotic diseases (Larchmont, N.Y.). ;16(3):205-11. PubMed PMID: 26824880; PubMed Central PMCID: PMC4779976.
Complete	Zhang T, Kedzierska-Mieszkowska S, Liu H, Cheng C, Ganta RR, Zolkiewski M. Aggregate-reactivation activity of the molecular chaperone ClpB from Ehrlichia chaffeensis. PloS one. ;8(5):e62454. PubMed PMID: 23667479; PubMed Central PMCID: PMC3646808.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

Obtained via FOIA by White Coat Waste Project

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	GANTA, ROMAN R.	BS,MS,PHD	PD/PI	EFFORT					NA
	N	Redacted by agreement	BS	Graduate Student (research assistant)						NA
	N		PHD	Staff scientist (Doctoral level)						NA
	N		MS, PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N		MS	Graduate Student (research assistant)						NA
	N		PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N		PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N		PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

Obtained via FOIA by White Coat Waste Project

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

Obtained via FOIA by White Coat Waste Project

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

Obtained via FOIA by White Coat Waste Project

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

Obtained via FOIA by White Coat Waste Project

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Kansas State University	929773554	KS-001	2 Fairchild Hall Manhattan KS 665061103

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

Obtained via FOIA by White Coat Waste Project

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Obtained via FOIA by White Coat Waste Project

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/08/2019

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 2R01AI070908-10
FAIN: R01AI070908

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. Chaffeensis Gene Expression

Paul R. Lowe
Kansas State University
PreAward Services
2 Fairchild Hall
1601 Vattier Street
Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Period Of Performance:
Budget Period: 07/10/2019 – 06/30/2020
Project Period: 12/15/2007 – 06/30/2024

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $536,311 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI070908. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Obtained via FOIA by White Coat Waste Project

Sincerely yours,

Gregory P. Smith
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Obtained via FOIA by White Coat Waste Project

SECTION I – AWARD DATA – 2R01AI070908-10

Award Calculation (U.S. Dollars)

Salaries and Wages	$187,481
Fringe Benefits	$59,994
Personnel Costs (Subtotal)	$247,475
Materials & Supplies	$76,448
Travel	$5,826
Other	$23,087
Federal Direct Costs	$352,836
Federal F&A Costs	$183,475
Approved Budget	$536,311
Total Amount of Federal Funds Obligated (Federal Share)	$536,311
TOTAL FEDERAL AWARD AMOUNT	$536,311
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$536,311

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
10	$536,311	$536,311
11	$536,311	$536,311
12	$536,311	$536,311
13	$536,311	$536,311
14	$536,311	$536,311

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908D
PMS Account Type:	P (Subaccount)
Fiscal Year:	2019

IC	CAN	2019	2020	2021	2022	2023
AI	8472364	$536,311	$536,311	$536,311	$536,311	$536,311

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C B / **OC**: 414B / **Released:** eRA Commons User Name 06/27/2019
Award Processed: 07/08/2019 12:02:56 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 2R01AI070908-10

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 2R01AI070908-10

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.

Obtained via FOIA by White Coat Waste Project

d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI070908. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:

Additional Costs

Obtained via FOIA by White Coat Waste Project

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

The budget period anniversary start date for future year(s) will be **July 1.**

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Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

Obtained via FOIA by White Coat Waste Project
~~~~~~~~~~

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Paula Acevedo
Email: paula.acevedo@nih.gov **Phone**: 301-435-2860 **Fax**: 301-493-0597

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 240-627-3341 **Fax**: 301-402-2508

SPREADSHEET SUMMARY
GRANT NUMBER: 2R01AI070908-10

INSTITUTION: KANSAS STATE UNIVERSITY

Budget	Year 10	Year 11	Year 12	Year 13	Year 14
Salaries and Wages	$187,481	$187,481	$187,481	$187,481	$187,481
Fringe Benefits	$59,994	$59,994	$59,994	$59,994	$59,994
Personnel Costs (Subtotal)	$247,475	$247,475	$247,475	$247,475	$247,475
Materials & Supplies	$76,448	$76,448	$76,448	$76,448	$76,448
Travel	$5,826	$5,826	$5,826	$5,826	$5,826
Other	$23,087	$23,087	$23,087	$23,087	$23,087
TOTAL FEDERAL DC	$352,836	$352,836	$352,836	$352,836	$352,836
TOTAL FEDERAL F&A	$183,475	$183,475	$183,475	$183,475	$183,475
TOTAL COST	$536,311	$536,311	$536,311	$536,311	$536,311

Facilities and Administrative Costs	Year 10	Year 11	Year 12	Year 13	Year 14
F&A Cost Rate 1	52%	52%	52%	52%	52%
F&A Cost Base 1	$352,836	$352,836	$352,836	$352,836	$352,836
F&A Costs 1	$183,475	$183,475	$183,475	$183,475	$183,475

Obtained via FOIA by White Coat Waste Project

PI: **GANTA, ROMAN R.**	Title: Vector and Host Contributions to the Regulation of E. Chaffeensis Gene Expression	
Received: 12/03/2018	FOA: PA18-484 Clinical Trial:Not Allowed	Council: 05/2019
Competition ID: FORMS-E	FOA Title: NIH Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
2 R01 AI070908-10	Dual:	Accession Number: 4243320
IPF: 4202801	Organization: KANSAS STATE UNIVERSITY	
Former Number:	Department: Diagnostic Medicne Pathobiolog	
IRG/SRG: ZRG1 IDM-V (02)M	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 10: 360,000 Year 11: 360,000 Year 12: 360,000 Year 13: 360,000 Year 14: 360,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: Evaluative Info HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Roman Ganta	Kansas State University	PD/PI
Redacted by agreement		Other (Specify)-Co-Investigator
		Other (Specify)-Co-Investigator
		Other (Specify)-Co-Investigator
		Other (Specify)-Co-Investigator

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
AI070908

b. Agency Routing Number

2. DATE SUBMITTED
2018-12-03

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 929773554

Legal Name*: Kansas State University
Department:
Division:
Street1*: 2 Fairchild Hall
Street2: 1601 Vattier Street
City*: Manhattan
County: Riley
State*: KS: Kansas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 66506-1103

Person to be contacted on matters involving this application
Prefix: Mr. First Name*: Paul Middle Name: R. Last Name*: Lowe Suffix:
Position/Title: Assoc Vice President/Research
Street1*: 2 Fairchild Hall
Street2: 1601 Vattier Street
City*: Manhattan
County: Riley
State*: KS: Kansas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 66506-1103
Phone Number*: 785-532-6804 Fax Number: 785-532-5944 Email: plowe@ksu.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-480771751-A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ❍ Resubmission
● Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Vector and Host Contributions to the Regulation of E. Chaffeensis Gene Expression

12. PROPOSED PROJECT
Start Date* 07/01/2019
Ending Date* 06/30/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
KS-001

Obtained via FOIA by White Coat Waste Project

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Roman Middle Name: Reddy Last Name*: Ganta Suffix:

Position/Title: Professor
Organization Name*: Kansas State University
Department: Diagnostic Medicne Pathobiolog
Division: College of Veterinary Medicine
Street1*: 336 Coles Hall
Street2: 1620 Denison Ave
City*: Manhattan
County: Riley
State*: KS: Kansas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 66506-5800
Phone Number*: 785-532-4612 Fax Number: 785-532-3939 Email*: rganta@vet.k-state.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$2,736,000.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$2,736,000.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Mr. First Name*: Paul Middle Name: R. Last Name*: Lowe Suffix:

Position/Title*: Assoc Vice President/Research
Organization Name*: Kansas State University
Department: PreAward Services
Division: Vice President for Research
Street1*: 2 Fairchild Hall
Street2: 1601 Vattier Street
City*: Manhattan
County: Riley
State*: KS: Kansas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 66506-1103
Phone Number*: 785-532-6804 Fax Number: 785-532-5944 Email*: plowe@ksu.edu

Signature of Authorized Representative*
Paul R. Lowe

Date Signed*
12/03/2018

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:CoverLetter1021468600.pdf

Obtained via FOIA by White Coat Waste Project

424 R&R and PHS-398 Specific
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Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Kansas State University
Duns Number:	929773554
Street1*:	2 Fairchild Hall
Street2:	1601 Vattier Street
City*:	Manhattan
County:	Riley
State*:	KS: Kansas
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	66506-1103
Project/Performance Site Congressional District*:	KS-001

Additional Location(s) File Name:

Obtained via FOIA by White Coat Waste Project

Funding Opportunity Number: PA-18-484. Received Date: 2018-12-03T17:42:09.000-05:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ● No

IACUC Approval Date: 09-12-2017

Animal Welfare Assurance Number A3609-01

3. Is proprietary/privileged information included in the application?* ● **Yes** ○ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	ProjectSummary1021468579.pdf
8. Project Narrative*	ProjectNarrative1021468561.pdf
9. Bibliography & References Cited	BibliographyReferencesCited1021468608.pdf
10.Facilities & Other Resources	FacilitiesOtherResources1021468563.pdf
11.Equipment	Equipment1021468564.pdf

Obtained via FOIA by White Coat Waste Project

PROJECT SUMMARY:

Rickettsial diseases caused by the Anaplasmataceae family pathogens of the genera *Ehrlichia* and *Anaplasma* remain a growing public health concern for over three decades and are also the second leading cause of tick- borne human infections in the USA and many parts of the world. Despite the complex cellular environments of arthropods and vertebrates having sophisticated systems of defense, the Anaplasmataceae pathogens evolved strategies to evade host clearance. Our prior studies demonstrated that the differential expression in vertebrate and tick cells alters host responses leading to variations in vertebrate host clearance/persistence. The central hypothesis of our competing renewal application remains that *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for pathogen's continued survival in vertebrate and tick cell environments. We have made substantial progress in addressing the three specific aims of the previous funded project. The progress from the prior funded cycle included numerous peer-reviewed publications, which paved the way for additional exciting new directions for this proposed competing renewal application. Goals set for the three new specific aims of this renewal application have a strong premise, as supported with published research results and with additional unpublished preliminary data. The extensive novel data generated with the previous R01 grant support formed a strong foundation for the following proposed three specific aims of this renewal application. 1) Characterize sequence-specific DNA binding proteins (DBPs) in *E. chaffeensis* impacting RNA polymerase function in support of understanding the pathogen's host-specific differential gene expression. 2) Characterize the functional significance of genes identified as essential for *E. chaffeensis in vivo* growth. 3) Mutagenesis and *in vivo* screening in defining genomic regions critical for the bacterial pathogenesis in vertebrate and tick hosts. The proposed specific aims are a logical extension of the substantial progress we have made during the previous funding cycle. The project goals have a strong scientific premise, as they are supported with the research data reported in many peer-reviewed publications and with the inclusion of additional preliminary data.

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PROJECT NARRATIVE

The results from this study will provide important information for understanding *Ehrlichia chaffeensis* pathogenesis, gene regulation and how the tick-transmitted pathogen escapes host response in vertebrates and how the loss of gene expression may impact its survival in ticks and vertebrates. This study will also allow us to determine how the tick transmitted pathogen continues to survive in ticks and animals, thus will aid in identifying targets for controlling the bacterial infections in people in the near future.

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FACILITIES OTHER RESOURCES

Laboratories: Dr. Ganta has three well-equipped and recently renovated research laboratories (450 sq. ft. each). Two laboratories have been designated for use in studies involving cell culture, nucleic acid and protein isolation and for setting up *in vitro* amplification procedures. The third laboratory is for conducting experiments where PCR product analysis, molecular cloning and protein analysis experiments, immunoblot and ELISA analysis are performed. Each laboratory has its own dedicated equipment items. Further, one laboratory is equipped to maintain tick colonies and has set up for providing 14 h day light and 10 h darkness. Chemicals, necessary reagents and consumables are maintained separately in each laboratory.

Offices: We have four 150 sq. ft. offices for use by the research personnel, including one occupied by Ganta and one office included office specialist to help with managing research needs such as to order chemicals and supplies and managing the resources well. In addition, the department also has a well-organized office constituting several secretarial staff and accountants to support the project needs.

Computers: Several personal computers are installed in all of the offices and in laboratories (every staff person has his/her own dedicated personal computer). All of the computers have Wi-Fi and Ethernet connections for the direct high-speed Internet access. We have a lifetime license for nucleic acid analysis software. Our college maintains a multimedia center and a technical support center which include many standby staff members to support the needs of faculty and staff.

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EQUIPMENT:

Major Equipment in my laboratory: The equipment in Dr. Ganta's laboratories include three electroporators, three conventional 96 tube capacity PCR machines (a Perkin-Elmer GenAmp 9700 and two Eppindorf temperature gradient PCR with an in situ PCR module), two Smart Cycler PCR systems and a BioRad iCycler system for real time PCR/RT-PCR analysis, manual and automated DNA sequencing systems, several agarose and poly acrylamide gel systems, a pulsed field gel apparatus, a 2D gel electrophoresis system for protein, DNA and RNA analysis, a hybridization oven, a blot transfer apparatus, a UV cross linker, Kodak image analysis system, an X-ray film developer, several water baths, shaker incubators, a speed-vac system, a sequencing gel drier system, several micro centrifuges, a sonicator, four CO2 incubators, two tri-gas incubators, two sterile cell-culture hoods, two cyto-centrifuges, a sero-centrifuge, two fume hoods, three cryogenic freezers, three chromatography refrigerators, three freezers, several liquid nitrogen tanks, several weighing balances (micro and macro), three refrigerated high speed centrifuges, a bench-top ultracentrifuge, and several pH meters. We are also equipped with two fluorescence microscopes with the capability to record digital images. Further, our college has a state of the art confocal microscopy facility and many molecular biology support instruments, such as bioanalyzers and imaging systems.

We also have the complete access to many shared facilities. They include several additional PCR machines, X-ray film developers, ice machines, scintillation counters, autoclaves, and a centralized deionized water supply. We also have a walk-in cold room, a 37 degree incubator, and an instrumentation room containing several pieces of commonly used equipment such as the high speed and ultra centrifuges. These are located within the close proximity of my laboratories. Our college also has a transmission and scanning electron microscope, a Becton Dickinson FACScan for flow cytometry analysis and maintains a core facility for confocal miscroscopic facility.

Kansas State University has several biotechnology core facilities to support research on genomics, functional genomics and molecular biology. The facilities include several state-of-the-art pieces of equipment, such as the MALDI-TOF and LC-MS/MS mass spectrometry instruments and protein/peptide sequencing equipment.

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Roman Middle Name Reddy Last Name*: Ganta Suffix:

Position/Title*: Professor
Organization Name*: Kansas State University
Department: Diagnostic Medicne Pathobiolog
Division: College of Veterinary Medicine
Street1*: 336 Coles Hall
Street2: 1620 Denison Ave
City*: Manhattan
County: Riley
State*: KS: Kansas
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 66506-5800

Phone Number*: 785-532-4612 Fax Number: 785-532-3939

E-Mail*: rganta@vet.k-state.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1987

Attach Biographical Sketch*: File Name: BiographicalSketch_Ganta1021468565.pdf

Attach Current & Pending Support: File Name:

Obtained via FOIA by White Coat Waste Project

Tracking Number: GRANT12753737 Funding Opportunity Number: PA-18-484 . Received Date: 2018-12-03T17:42:09.000-05:00

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

Redacted by agreement

Obtained via FOIA by White Coat Waste Project

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

Redacted by agreement

Obtained via FOIA by White Coat Waste Project

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Roman R. Ganta**

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Professor and Director, Center of Excellence for Vector-Borne Diseases, Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Andhra University, Waltair, India	B. S.	05/1978	Zoology, Botany, Chemistry
Andhra University, Waltair, India	M.S.	05/1980	Biochemistry
All India Institute of Medical Sciences, New Delhi, India	Ph.D.	03/1987	Biochemistry
The Scripps Research Institute, La Jolla, CA	Postdoctoral	03/1990	Molecular Biology
University of Florida, Gainesville, FL	Postdoctoral	03/1992	Molecular Parasitology

A. Personal Statement

I have 28 years of research experience on tick-borne diseases, including studying human ehrlichiosis, anaplasmosis and Rocky Mountain spotted fever. I have the necessary expertise and experience to serve as the PI for the proposed competing renewal application. I have broad experience in carrying out independent and collaborative research and administering many federally funded grants. I also have a broad administrative experience in managing a center of excellence; the Center of Excellence for Vector-Borne Diseases (CEVBD) at Kansas State University (KSU). I am the founder of this center, which was established three years ago with a vision to expand the research on various tick and other vector-borne diseases. I have been serving as the founding Director of the center. I also have a broad research background in molecular biology, molecular microbiology, immunology, and biochemistry of vector-borne diseases, with specific training and expertise in tick- and mosquito-borne diseases bacterial, parasitic and viral diseases. Our studies involve assessing pathogen molecular structure, host response assessment, the effect of vector and host cell environments on the pathogen survival, pathogenesis, gene regulation and in developing molecular diagnostic tests. With funding received as the PI on several NIH-funded grants and from various federal, state, university, foundation and industry support, I continue to carryout independent and collaborative research with many scientists at K-State and from other academic institutions. I am also active as an NIH reviewer for many study sections since 2003. My years of experience as an independent scientist, administrator and as a mentor, prepared me well in recognizing challenges of a research program and in troubleshooting in a timely manner.

B. Positions and Honors

Positions; past and current:

1992-98	Assistant Scientist, Dept. Pathobiology, University of Florida, Gainesville, FL
1998-03	Assistant Professor, Department of Diagnostic Medicine/Pathobiology, Kansas State University
2003-07	Associate Professor, Department of Diagnostic Medicine/Pathobiology, Kansas State University
2007-current	Professor, Department of Diagnostic Medicine/Pathobiology, Kansas State University
2005-13	Director, Molecular Biology Core, Center Of Biomedical Research Excellence (COBRE) Epithelial Function in Health and Disease at CVM, KSU
2015-current	Director, Center of Excellence for Vector-Borne Diseases, Department of Diagnostic Medicine/Pathobiology, Kansas State University

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Grant review experience:

Serving on the National Institutes of Health (NIH) study sections, USA since 2003:

1) Chairperson, ZRG1 IDM S (81) study section to review R15 (academic research enhancement award, AREA) applications (2016)
2) Member of the Vector Biology (VB) Study Section (4 year term; 2006-2010). (Served as a Co-Chair at June, 2009 panel meeting)
3) Ad-hoc member on the Infectious Diseases and Microbiology (IDM) Study Sections (but I am called on a regular basis, as this panel doesn't host regular members) (2005, 2009-2018)
4) Ad-hoc member on a Special Study Section for the RFA, AI-11-014 titled 'Partnerships for Biodefense' (2011)
5) Ad-hoc member on the Host Interaction with Bacterial Pathogens (HIBP) Study Section (2004, 2018).
6) Ad-hoc member on the Bacteriology and Mycology 1 (BM1) Study Section (2003).

Other major grant review experiences:

2002-17 Oklahoma Center for the Advancement of Science and Technology, Oklahoma City, OK Grants Review panel (including serving as a chairperson for couple of years)
2013-18 Military Infectious disease Research Program (MIDRP), USA: Review panel member

Professional memberships/affiliations:

1998-current Member, American Society for Rickettsiology
2003-07 Secretary/Treasurer, American Society for Rickettsiology
2007-09 Vice-President, American Society for Rickettsiology
2009-10 President, American Society for Rickettsiology
2001-current Member, American Society for Microbiology
2008-current Past-President (2016), President (2015), Vice-President (2014) and Council Member (2010-2013) and Vector-borne and Parasitic diseases Section Leader (2008-2017), Conference of Research Workers in Animal Diseases (CRWAD), USA

Awards:

2005 Pfizer Animal Health Award for Research Excellence, College of Veterinary Medicine, KSU
2012 Recipient of the title 'Fellow of the Association of Biotechnology and Pharmacy (FABAP)', India: This is a prestigious award bestowed only to a very few nationally and internationally recognized scientists by the organization, the Association of Biotechnology and Pharmacy, India.

Editorial board service:

2009-current Editorial Board: Current Trends in Biotechnology and Pharmacy (ISSN 0973-8916)
2010-current Regional Editor: Research Journal of Microbiology (ISSN: 1816-4935)
2011-current Editorial Board member, Frontiers in Microbiology
2011-current Academic Editor, PLoS ONE
2017-current Editorial Board member, Genomics

C. Contribution to Science

Ehrlichia chaffeensis, a tick-borne rickettsial organism, causes the disease, human monocytic ehrlichiosis. The pathogen also causes disease in several other vertebrates, including dogs and deer. We have active research program established at Kansas State University. We made many important discoveries over the years. The following are a few, which we consider as the most significant contributions in advancing the understanding the pathogenesis and in developing methods of prevention.

1) We reported the methods describing mutagenesis in *E. chaffeensis*. We paved the way for opening novel methods for other investigators to carry out similar research in this pathogen and other related pathogens causing diseases in various vertebrate animals, including humans. I conceived the research project, obtained funding support and oversaw all aspects of the project goals. The description of the mutants and their further characterization were published in the following four articles:

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a) Targeted and random mutagenesis of *Ehrlichia chaffeensis* for the identification of genes required for in vivo infection. Cheng C, Nair AD, Indukuri VV, Gong S, Felsheim RF, Jaworski D, Munderloh UG and **Ganta RR**. PLoS pathogens. 2013; 9(2):e1003171. PMID: 23459099; PMCID: PMC3573109

b) Mutations in *Ehrlichia chaffeensis* causing polar effects in gene expression and differential host specificities. Cheng C, Nair ADS, Jaworski DC, and **Ganta RR**. PLoS ONE (2015 Jul 17;10(7):e0132657. doi: 10.1371/journal.pone.0132657. eCollection 2015. PMID: 26186429; PMCID: PMC4505860

c) A genetic system for targeted mutations to disrupt and restore genes in the obligate bacterium, Ehrlichia chaffeensis. Wang Y, Wei L, Liu H, Cheng C, **Ganta RR**. Sci Rep. 2017 Nov 17;7(1):15801. doi: 10.1038/s41598-017-16023-y. PMID: 29150636; PMCID: PMC5693922

d) Impact of Three Different Mutations in *Ehrlichia chaffeensis* in altering the global gene expression patterns. Kondethimmanahalli C, **Ganta R.** Sci Rep. 2018 Apr 18;8(1):6162. doi: 10.1038/s41598-018-24471-3. PMID: 29670161; PMCID: PMC5906474

2) We are also the first in demonstrating that the mutations at certain genes result in *E. chaffeensis* rapid clearance. We reasoned that the attenuated mutants could be valuable in serving as live vaccines to confer protection against experimental infection challenge. We investigated three mutant strains and reported the data in the following peer-reviewed manuscripts.

a) Attenuated Mutants of *Ehrlichia chaffeensis* in Inducing Protection against Wild-type Infection Challenge in the Reservoir Host and in an Incidental Host. Nair AD, Cheng C, Jaworski DC, Ganta S, Sanderson MW, and **Ganta RR**. *Infect. Immun*. 2015; PMID: 25916990; PMCID: PMC4468539

b) Vaccination with an Attenuated Mutant of Ehrlichia chaffeensis Induces Pathogen-Specific CD4+ T Cell Immunity and Protection from Tick-Transmitted Wild-Type Challenge in the Canine Host. McGill JL, Nair AD, Cheng C, Rusk RA, Jaworski DC, **Ganta RR**. *PLoS One*. 2016 Feb 3;11(2):e0148229. doi: 10.1371/journal.pone.0148229. eCollection 2016. PMID: 26841025; PMCID: PMC4739596

c) Antigen-Specific CD4+CD8+ Double-Positive T Cells Are Increased in the Blood and Spleen During *Ehrlichia chaffeensis* Infection in the Canine Host. McGill JL, Wang Y, Ganta CK, Boorgula GDY, **Ganta RR**. *Front Immunol.* 2018 Jul 11;9:1585. doi: 10.3389/fimmu.2018.01585. eCollection 2018. PMID: 30050533; PMCID: PMC6050357

3) We are the first group to develop novel molecular tools in support of studying the regulation of gene expression for an intraphagosomal pathogen for which a transformation system is not available and genetic tools are at infancy. We set a novel trend to initiate a new research direction. The following are few publications outlining our contributions.

a) Transcription of *Ehrlichia chaffeensis* genes is accomplished by RNA polymerase holoenzyme containing either sigma 32 or sigma 70. Liu H, Von Ohlen T, Cheng C, Faburay B, **Ganta RR**. *PloS One*. 2013; 8(11):e81780. PMID: 24278458 PMCID: PMC3836757

b) Sequence determinants spanning -35 motif and AT-rich spacer region impacting Ehrlichia chaffeensis Sigma 70-dependent promoter activity of two differentially expressed p28 outer membrane protein genes. Liu H, Jakkula LU, Von Ohlen T, Ganta RR. *DNA Res*. 2016 Jul 8. pii: dsw034. [Epub ahead of print] PMID: 27402867; PMCID: PMC5066175

URL to a full list of my published work in a publicly available digital database, My Bibliography: https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/48025032/?sort=date&direction=descending

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D. Research Support

Ongoing Research Support

R01 AI070908A (Ganta R) 08/01/2014-07/31/2019 (now in no-cost extension)
NIH NIAID
Vector and host contributions to the regulation of *E. chaffeensis* gene expression

The major goals of this project are to evaluate the contributions of vector and host cell environment in altering the pathogen gene expression.

Private Source 11/01/2016-10/31/2020
Pathogenesis of Heartwater disease

The goals of this project are to establish a heartwater disease project to study Ehrlichia ruminantium pathogenesis and vaccine development to protect ruminants against this important foreign animal disease.

Private Source 03/01/2018 - 02/29/2020
Antibody assessment of Ehrlichia, Anaplasma and Borrelia species infections in dogs

The major goals of this project are to assess the antibody prevalence in dogs against tick-borne pathogens of the genera Anaplasma, Ehrlichia and Borrelia.

Private Source 11/01/2017-04/30/2019
Evaluation of clinical protection afforded by prototypes vaccines against *Ehrlichia canis* infection.

The objective of this study is to assess the vaccine potential of recombinant vaccines against Ehrlichia species infections in the canine challenge model.

Private Source 10/25/2018-10/24/2019
Modifying Ehrlichia attenuated vaccine constructs

The objective of this study is to improve the Ehrlichia species attenuated vaccine constructs for use in developing vaccines suitable for protecting canine host against tick-transmitted Ehrlichia infections.

Completed Research Support

5P20 GM103638-04 (McGill J) 07/01/2015 – 06/30/2017
University of Kansas Medical Center Research Institute/NIH (Mentor – Ganta R)
Alternative functions for gamma delta T cells in the immune response to Mycobacterium

The major goal of this project is to conduct transcriptomics analysis on γδ T cells purified from the blood and tissues of cattle infected with Mycobacterium bovis, to identify alternative functions for nonconventional.

5P20 GM103418-15 (Zolkiewski M) 05/01/2015-04/30/2016
NIH NIGM
KINBRE-Bridging award – Molecular chaperones of Ehrlichia chaffeensis
Role on the Project for Ganta – Co-PI

The major goals of this project are to generate preliminary data required to be competitive at the NIH for R01 funding. The R01 application is currently under review with specific goal to characterize the biological activity and the biochemical mechanism of the molecular chaperone ClpB in *E. chaffeensis*.

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Page 291 of 430 to Page 302 of 430

Withheld pursuant to exemption

Redacted by agreement

of the Freedom of Information and Privacy Act

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 929773554

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Kansas State University

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Bas Salary	EFFORT			47,400.00	15,168.00	62,568.00
2 .	Dr.	Redacted by agreement				Co-Investigator					22,222.00	7,111.00	29,333.00
3 .	Dr.					Co-Investigator					13,666.00	4,373.00	18,039.00
4 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00
5 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **189,140.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12			48,000.00	15,360.00	63,360.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**63,360.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**252,500.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

Funding Opportunity Number: PA-18-484 . Received Date: 2018-12-03T17:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period:** 1

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,944.00
2. Foreign Travel Costs	
Total Travel Cost	**5,944.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	78,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition remission	0.00
9 . Animal Care	23,556.00
Total Other Direct Costs	**101,556.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**360,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On campus Organized Research MTDC rate	52	360,000.00	187,200.00
		Total Indirect Costs	**187,200.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US Department of Health and Human Services, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**547,200.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	547,200.00

L. Budget Justification* File Name: BudgetJustification1021468580.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 929773554

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Kansas State University

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Base Salary	EFFORT			47,400.00	15,168.00	62,568.00
2 .	Dr.	Redacted by agreement				Co-Investigator					22,222.00	7,111.00	29,333.00
3 .						Co-Investigator					13,666.00	4,373.00	18,039.00
4 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00
5 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **189,140.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12			48,000.00	15,360.00	63,360.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**63,360.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**252,500.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

Funding Opportunity Number: PA-18-484 . Received Date: 2018-12-03T17:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,944.00
2. Foreign Travel Costs	
Total Travel Cost	**5,944.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	78,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition remission	0.00
9 . Animal Care	23,556.00
Total Other Direct Costs	**101,556.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**360,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On campus Organized Research MTDC rate	52	360,000.00	187,200.00
		Total Indirect Costs	**187,200.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US Department of Health and Human Services, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**547,200.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	547,200.00

L. Budget Justification* File Name: BudgetJustification1021468580.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 929773554

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Kansas State University

Start Date*: 07-01-2021 **End Date*:** 06-30-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Base Salary	EFFORT			47,400.00	15,168.00	62,568.00
2 .	Dr.	Redacted by agreement				Co-Investigator					22,222.00	7,111.00	29,333.00
3 .						Co-Investigator					13,666.00	4,373.00	18,039.00
4 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00
5 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **189,140.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12			48,000.00	15,360.00	63,360.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**63,360.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**252,500.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

Funding Opportunity Number: PA-18-484 . Received Date: 2018-12-03T17:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2021 **End Date*:** 06-30-2022 **Budget Period:** 3

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,944.00
2. Foreign Travel Costs	
Total Travel Cost	**5,944.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2021 **End Date*:** 06-30-2022 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	78,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition remission	0.00
9 . Animal Care	23,556.00
Total Other Direct Costs	**101,556.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**360,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On campus Organized Research MTDC rate	52	360,000.00	187,200.00
		Total Indirect Costs	**187,200.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US Department of Health and Human Services, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**547,200.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	547,200.00

L. Budget Justification* File Name: BudgetJustification1021468580.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 929773554

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Kansas State University

Start Date*: 07-01-2022 **End Date*:** 06-30-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Base Salary	EFFORT			47,400.00	15,168.00	62,568.00
2 .	Dr.	Redacted by agreement				Co-Investigator					22,222.00	7,111.00	29,333.00
3 .						Co-Investigator					13,666.00	4,373.00	18,039.00
4 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00
5 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **189,140.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12			48,000.00	15,360.00	63,360.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**63,360.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**252,500.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

Funding Opportunity Number: PA-18-484 . Received Date: 2018-12-03T17:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2022 **End Date*:** 06-30-2023 **Budget Period:** 4

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,944.00
2. Foreign Travel Costs	
Total Travel Cost	**5,944.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2022 **End Date*:** 06-30-2023 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	78,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition remission	0.00
9 . Animal Care	23,556.00
Total Other Direct Costs	**101,556.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**360,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On campus Organized Research MTDC rate	52	360,000.00	187,200.00
		Total Indirect Costs	**187,200.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US Department of Health and Human Services, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**547,200.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	547,200.00

L. Budget Justification* File Name: BudgetJustification1021468580.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 929773554

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Kansas State University

Start Date*: 07-01-2023 **End Date*:** 06-30-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Roman	Reddy	Ganta		PD/PI	Institutional Base Salary	EFFORT			47,400.00	15,168.00	62,568.00
2 .	Dr.	Redacted by agreement				Co-Investigator					22,222.00	7,111.00	29,333.00
3 .						Co-Investigator					13,666.00	4,373.00	18,039.00
4 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00
5 .	Dr.					Co-Investigator					30,000.00	9,600.00	39,600.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **189,140.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12			48,000.00	15,360.00	63,360.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**63,360.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**252,500.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

Funding Opportunity Number: PA-18-484 . Received Date: 2018-12-03T17:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2023 **End Date*:** 06-30-2024 **Budget Period:** 5

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,944.00
2. Foreign Travel Costs	
Total Travel Cost	**5,944.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 929773554
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Kansas State University

Start Date*: 07-01-2023 **End Date*:** 06-30-2024 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	78,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition remission	0.00
9 . Animal Care	23,556.00
Total Other Direct Costs	**101,556.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**360,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On campus Organized Research MTDC rate	52	360,000.00	187,200.00
		Total Indirect Costs	**187,200.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US Department of Health and Human Services, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**547,200.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	547,200.00

L. Budget Justification* File Name: BudgetJustification1021468580.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Obtained via FOIA by White Coat Waste Project

BUDGET JUSTIFICATION

Personnel

Roman R. Ganta, Ph.D., PI EFFORT He will be responsible for overseeing all aspects of the project. A microbiologist Redacted by agreement three scientists Redacted by agreement Redacted having experience working on the project and a post-doctoral associate (to be identified) will be part of the project and they will be under Ganta's supervision, although they are well trained to be executing most of the proposed experiments. The research team meets once every week to discuss progress and updates to ensure the successful execution of the project, including discussing data and preparing manuscripts for publications. Further, the team will discuss recent publications each week to stay up to date with the current research advances.

Redacted by agreement EFFORT , Redacted by agreement EFFORT Redacted by agreement (EFFORT and Redacted by agreement Redacted by agreement EFFORT (Note: additional support for these scientists come from state, university, or foundation support at the Center of Excellence for Vector-Borne Diseases (CEVBD). These four members will oversee all aspects of molecular biology, mutagenesis, animal and tick infection studies and work closely with the PI, post-doc EFFORT and other members of the research team. They will also be actively involved in weekly project meetings to discuss the research progress, data analysis and in preparing manuscripts. Redacted will lead mostly on gene regulation aspects, Redacted by agreement will focus on mutagenesis experiments, while Redacted by agreement will be involved in animal and tick experiments. All listed members contributed greatly in generating the preliminary data and are well qualified to carry out the goals as proposed. A post-doctoral associate will be employed to receive research training and to perform experiments proposed in the project.

Supplies

Average monthly costs is $6,500 to obtain media reagents, sterile pipets and flasks, culture plates, pipets, gloves, antibody prep costs, PCR and centrifuge tubes, molecular biology reagents and kits, radio active, other chemicals and for the purchase of animals.

Travel

Travel funds are requested for the PI and appropriate personnel to attend a scientific conference(s) to present and obtain feedback on research progress ($5,944 per year).

Other Direct Costs

Animal per diem costs will be $15,680 per year ($8/day/dog x 70 days x 28 dogs) and remaining funds will be used for blood collection, CBC analysis and pathology services.

Total budget cap:

The total was only increased by 20%compared to prior funded project as per the NIAID cap amount. The additional budget request is also justified to keep up the increased personnel costs and the steady increase of supplies cost and other costs outlined above.

Obtained via FOIA by White Coat Waste Project

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		945,700.00
Section B, Other Personnel		316,800.00
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		1,262,500.00
Section C, Equipment		
Section D, Travel		29,720.00
1. Domestic	29,720.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		507,780.00
1. Materials and Supplies	390,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2	117,780.00	
10. Other 3		
Section G, Direct Costs (A thru F)		1,800,000.00
Section H, Indirect Costs		936,000.00
Section I, Total Direct and Indirect Costs (G + H)		2,736,000.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		2,736,000.00

Obtained via FOIA by White Coat Waste Project

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

Obtained via FOIA by White Coat Waste Project

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ● Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ● Yes ○ No

5. Change of Investigator/Change of Institution Section

☐ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Grantee Institution

*Name of former institution:

Obtained via FOIA by White Coat Waste Project

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	SpecificAims1021468609.pdf
3. Research Strategy*	ResearchStrategy1021468607.pdf
4. Progress Report Publication List	ProgressReportPublicationsList1021468606.pdf
Other Research Plan Section	
5. Vertebrate Animals	VertebrateAnimals1021468602.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	
10. Resource Sharing Plan(s)	ResourceSharingPlan1021468603.pdf
11. Authentication of Key Biological and/or Chemical Resources	AuthenticationOfResources1021468581.pdf
Appendix	
12. Appendix	

Obtained via FOIA by White Coat Waste Project

Funding Opportunity Number: PA-18-484. Received Date: 2018-12-03T17:42:09.000-05:00

SPECIFIC AIMS: Rickettsial diseases caused by the *Anaplasmataceae* family pathogens of the genera *Ehrlichia* and *Anaplasma* remain a growing public health concern spanning the past three decades, and are also the second leading cause of tick-borne infections in humans throughout the USA and many parts of the world. The diseases include human monocytic and granulocytic ehrlichiosis caused by *Ehrlichia chaffeensis* and *Ehrlichia ewingii* respectively, and human granulocytic anaplasmosis resulting from *Anaplasma phagocytophilum* infections. Recently, another tick-borne pathogen, *Ehrlichia muris* subsp. *eauclairensis*, has been identified as causing another ehrlichial disease in people. Despite the complex cellular environments of arthropods and vertebrates having sophisticated systems of defense, these pathogens have evolved strategies to evade host clearance. For example, *E. chaffeensis* in macrophages and tick cells has distinct patterns of protein expression and the pathogen translocates effectors in altering host responses to support its continued survival in a host. Our prior studies demonstrated that differential expression in vertebrate and tick cells alters host responses leading to variations in vertebrate host clearance/persistence. The *central hypothesis* of our competing renewal application remains that *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for the pathogen's continued survival in vertebrate and tick cell environments. Specific aims of the previous funded project are to 1) characterize the *E. chaffeensis* RNA polymerase complex in understanding the pathogen's host-specific differential gene expression; 2) evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for the *in vivo* growth of *E. chaffeensis*; and 3) perform mutational analysis and *in vivo* screening to identify additional genes essential for *E. chaffeensis* pathogenesis in vertebrate and tick hosts. We made substantial progress in addressing all three specific aims. The progress, as evidenced in numerous peer-reviewed publications (24 peer-reviewed publications and 7 reviews/book chapters), paved the way for new directions of this renewal application. The new specific aims of this renewal application continue to address the above stated hypothesis. The proposed aims are supported with a strong premise. Furthermore, the proposed project goals are highly innovative and attempt to fill-in a significant gap of knowledge. We are the only research group investigating the detailed regulation of gene expression in *Ehrlichia* species and such studies may be extended in other *Ehrlichia* and *Anaplasma* species pathogens. We also lead the investigations on targeted mutagenesis studies for *Anaplasmataceae* pathogens, including in creating mutations disrupting and restoring a gene function. We used the methods in studies to understand pathogenesis and in studying gene regulation in *Ehrlichia* species. Targeted mutations to disrupt and also to functionally restore a gene will be valuable in testing molecular Koch's postulates. We have also created targeted mutations to generate bacterial fusion proteins, which are important in studies to characterize *E. chaffeensis* proteins *in vivo*. Additionally, we developed physiologically relevant animal infection models. Moreover, we standardized tick transmission methods in support of understanding the bacterial pathogenesis, host responses and vaccine development. We also extended studies in developing an attenuated vaccine to protect an incidental host from *E. chaffeensis* by needle and tick transmitted challenge. The *in vivo* infection models will also be valuable in investigating the importance of genes to the *E. chaffeensis* life cycle in tick and vertebrate hosts. Collectively, the extensive novel data, generated with the support of our previous R01 grant, form a strong foundation for the proposed three specific aims of this renewal application.

Specific Aims:

1) Characterize sequence-specific DNA binding proteins (DBPs) in *E. chaffeensis* impacting RNA polymerase function in support of understanding the pathogen's host-specific differential gene expression.
2) Characterize the functional significance of genes identified as essential for *E. chaffeensis in vivo* growth.
3) Mutagenesis and *in vivo* screening in defining genomic regions critical for the bacterial pathogenesis in vertebrate and tick hosts.

The proposed specific aims have a strong scientific premise and are a logical extension of the substantial progress we made during the previous funding cycle. The project goals are supported by the research data reported in our several peer-reviewed publications and with the inclusion of additional data. We have sufficient expertise to ensure successful execution of the project, and we included well thought-out authentication plans. All molecular tools to study gene expression were developed by us. We also have accomplished mutagenesis and *in vivo* screening methods to test the importance of *E. chaffeensis* genes in tick and vertebrate hosts. We believe that the goals of this renewal project are also critical in advancing understanding of the *Ehrlichia* pathogenesis. We expect that our continued progress will pave the way for similar studies in related rickettsials in controlling diseases caused by *Anaplasma, Ehrlichia* and *Neorickettsia* species.

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SIGNIFICANCE: *Anaplasmataceae* family pathogens belonging to the order Rickettsiales are obligate, intracellular, Gram-negative bacteria, which include the genera *Ehrlichia, Anaplasma* and *Neorickettsia* [1]. A steady increase of potentially fatal human diseases caused by the species of the genera *Ehrlichia* and *Anaplasma* has been reported during the last three decades [2-5]. These include three tick-transmitted emerging diseases in people caused by *Ehrlichia* species: human monocytic ehrlichiosis (HME) caused by *E. chaffeensis* [6, 7], human ewingii ehrlichiosis caused by *E. ewingii* [8], and more recently identified infections with *E. muris* subsp. *eauclairensis* [5]. Also, *Anaplasma phagocytophilum* is responsible for causing human granulocytic anaplasmosis (HGA) [9, 10]. Tick-transmitted pathogens must have strategies to persist in both tick and vertebrate hosts in order to successfully complete their infection cycle. Unlike insect vectors, the probability of transmitting a pathogen from a tick to a vertebrate host and similarly from a vertebrate host to a tick is significantly lower. Thus, it is necessary for tick-transmitted pathogens to have evolved strategies in establishing persistent infections in both vertebrate and tick hosts. Indeed, it is widely documented that tick-transmitted Rickettsiales of the genera *Ehrlichia* and *Anaplasma*, including *E. chaffeensis,* cause persistent infections in a wide range of vertebrate hosts [11-20].

An infectious disease is the result of a pathogen's ability to overcome host clearance. It is important to determine how pathogens overcome a host challenge in support of completing their transmission cycle. Recent research suggests that *Anaplasmataceae* pathogens express several proteins on their cell surface and secrete some proteins into the host cell cytoplasm, nucleus and mitochondria for their survival and growth in the host cell environments [21-24]. Our group and other investigators have presented evidence that *Ehrlichia* species alter the expression of many proteins in a host cell-specific manner when replicating in vertebrate and invertebrate host cells [20, 25-29]. Our previous studies also suggest that the major expression from the p28-Omp multi-gene locus from *E. chaffeensis* differs in vertebrate and tick cells [28]. The p28-Omp antigens may be essential components for the pathogen's survival by adapting to multiple hosts and to establish persistent infection, as these antigens are the most abundant in a host's induction of antibody responses [30, 31]. Adoptive transfer of antibodies against the p28-Omp antigens of *E. chaffeensis* protects against fatal infections in the SCID mouse model [32]. Prior studies also suggest that these membrane expressed proteins function as porins [33, 34]. The clearance of *E. chaffeensis* by a vertebrate host is delayed when it is grown in tick cells as compared with those grown in vertebrate host cells [35, 36]. Furthermore, we demonstrated that the host response differs considerably for *E. chaffeensis* originating from tick or tick cells compared to vertebrate host environments [35, 36]. Our proposed research has the primary focus of gaining fundamental knowledge important for the *in vivo* growth of *E. chaffeensis* by taking advantage of the newly developed methods for creating targeted mutagenesis and in defining the regulation of genes [37, 38]. The advancements we anticipate from the proposed goals of this competing renewal application will be important in devising methods of controlling tick-borne diseases caused by *Anaplasmataceae* pathogens.

INNOVATION: Much of the fundamental knowledge about how host-specific gene expression is accomplished and how it contributes to the pathogen's persistence can be understood by studying the regulation of gene expression. Likewise, defining the importance of pathogen proteins towards growth and pathogenesis of an organism requires genetic manipulation systems for creating knockouts or knockdowns of protein expression. The goals we set and accomplished during the previous R01 funding cycle were highly innovative and also highly challenging. Nevertheless, we have made substantial progress in accomplishing the proposed goals. For example, we are the only group in the rickettsial field developing methods to study regulation of gene expression, which include the development of an *in vitro* transcription system and the use of *E. coli* surrogate systems; our methods offer an innovative means to understand how *E. chaffeensis* and other related pathogens regulate gene expression. In the absence of a fully established genetic tool kit, such as the ability to perform transgene expression studies, defining a gene function using the *E. coli* surrogate system offers novel ways for understanding the significance of the pathogen's differential gene expression and to study transcription of the pathogen genes. Although similar methods are used to study gene regulation in other obligate intracellular pathogens for which mutational methods are also limited, *Chlamydia* species [39, 40], we are the first and only group studying regulation of gene expression in Rickettsiales.

Another challenging, highly sought after and innovative goal, of our previous funding cycle was to investigate the development of targeted mutagenesis methods. We have made substantial progress. Our research team has accomplished the development of targeted mutagenesis methods, which we believe will facilitate investigations by other researchers in the *Anaplasmataceae* field. We developed methods to create mutations in *Ehrlichia* species to both disrupt and restore a gene function. We also created an *E. chaffeensis* transposon mutant library, which included many novel mutants. Further, we developed animal and tick challenge models and utilized them in mapping the impact of mutations on the pathogen's growth in vertebrate and tick hosts and for vaccine development. We will take advantage of all these novel research tools in this renewal project.

PROGRESS REPORT FOR THE PREVIOUS FUNDING CYCLE: The previous funding cycle from 08/09/2013 to 07/31/2018 (a 5 year project and now in a one year no cost extension) included three specific aims; we published 24 peer-reviewed manuscripts and 7 book chapters/review articles. Thirteen manuscripts were the direct result of research proposed (summarized below). Although not summarized, 10 additional manuscripts were also related to the previous supported project (included in the 'Progress Report Publications' section).

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We also have Unpublished in progress currently. Further, we PATENT PENDING PATENT PENDING Our research is well recognized, as also evidenced by frequent invitations to present at scientific meetings and academic institutions (Ganta gave 26 invited talks between 2014-2018).

Specific Aim 1) Characterize *E. chaffeensis* RNA polymerase (RNAP) complex in support of understanding the pathogen's host-specific differential gene expression: This is a very complex and challenging goal. Like in other obligate bacteria, most of the RNAP characterization could only be performed *in vitro* and using novel methods, such as an *E. coli* surrogate system. Currently, gene regulation in *E. chaffeensis* and related pathogens in tick and vertebrate hosts is poorly understood. We published **four** manuscripts and have Unpublished the following summaries describe the progress for this aim.

Liu et al., 2013: In prokaryotes, transcription is controlled by transcription initiation factors (sigma factors), which bind to RNAP core enzyme and making it a promoter-specific holoenzyme [41]. *E. chaffeensis* contains only two sigma factor genes (*rpoH* and *rpoD*) encoding for σ^{32} (stress response) and σ^{70} (housekeeping) factors. Studying gene regulation in *E. chaffeensis* is challenging due to lack of a transformation system. We developed *in vitro* transcription and *E. coli*-based surrogate transcription system to study *E. chaffeensis* gene regulation. We used the *E. coli* strain; CAG57101 where its native σ^{32} is knocked out, respectively. In their place, *E. chaffeensis* σ^{32} was expressed from a plasmid to functionally complement *E. coli* RNAP, thus making the *E. coli* strain specific in driving transcription from *E. chaffeensis* genes recognized by σ^{32}. We mapped transcription start sites and evaluated promoter regions of several genes. We also identified *E. chaffeensis* RNAP binding motifs (-10 and -35) for several genes. We reported that the -10 and -35 motifs of σ^{32} and σ^{70} share extensive similarities and that *E. chaffeensis* transcription can be accomplished by either one of the sigma factors with varying affinities. We also showed that the σ^{32} and σ^{70} transcripts are higher during the early replicative stage and their expression was similar for the entire replication cycle. We also established that the -35 motif is essential for σ^{70}. We provided the first evidence that *E. chaffeensis* gene regulation is unique in utilizing either σ^{32} or σ^{70} in transcribing a gene.

Liu et al., 2016: We performed detailed mapping of the RNAP binding motifs of promoter regions of two *E. chaffeensis* immunogenic outer membrane protein (p28-Omp) genes (ECH_1136 and ECH_1143). In this study, we used *E. coli*-based surrogate transcription system to map *E. chaffeensis* σ^{70} recognized genes. An *E. coli* strain; CAG20177 was used where its native σ^{70} is repressed and *E. chaffeensis* σ^{70} was expressed from a plasmid to functionally complement *E. coli* RNAP. This allowed the *E. coli* strain to be specific in driving transcription from *E. chaffeensis* σ^{70} genes. These two genes were selected as they are differentially expressed in tick and vertebrate hosts and that they are primarily transcribed with σ^{70}. We mapped -10 and -35 motifs and the AT rich spacers in defining the critical determinants of RNAP binding. Numerous point mutations spanning the entire -35 motif were carried out; the first three nucleotides of the -35 motif, TTG, are extensively conserved in various *E. chaffeensis* genes. TTG appears to be vital for σ^{70} function. Further, we reported that the variations in AT-rich spacer sequence contribute to promoter-specific differences in gene expression. *We then mapped σ^{70} protein domains critical for interacting with -10 and -35 motifs of the two* gene *promoters.* This is the first detailed description of gene mapping in *Anaplasmataceae* family pathogens.

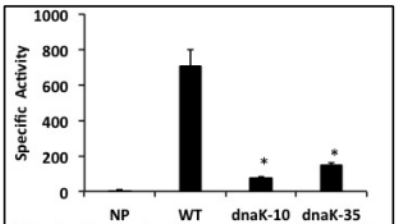

Fig 1. β-galactosidase activity driven by *E. chaffeensis* dnaK promoter in the *E. coli* (CAG57101) with *E. chaffeensis* σ^{32} expression; wild-type (WT) and with -10 and -35 motif deletions (dnaK-10 and dnaK-35). NP, no promoter control.

Unpublished *(manuscript in progress):* We have also mapped the promoter region of the *dnaK* gene (ECH_0471), which is primarily transcribed by σ^{32}. We used the previously described *E. coli* strain CAG57101 expressing *E. chaffeensis σ^{32} protein to map dnaK.* We cloned the *dnaK* promoter in front of a reporter gene (β-galactosidase) in a plasmid and introduced this into CAG57101. *DnaK* -10 and -35 motifs were then mapped (Fig. 1). Deleting -10 or -35 motifs caused significant reduction in β-galactosidase activity compared to the wild type ($p \leq 0.0002$), that is distinct from σ^{70} genes where only -35 motif is required [42]. We then performed detailed point mutation analysis to define the entire -10 motif (Fig. 2). Changes in the first 5 positions of -10 motif resulted in a significant drop in promoter activity. *We also mapped the AT rich spacer sequence (Fig. 3). Two*

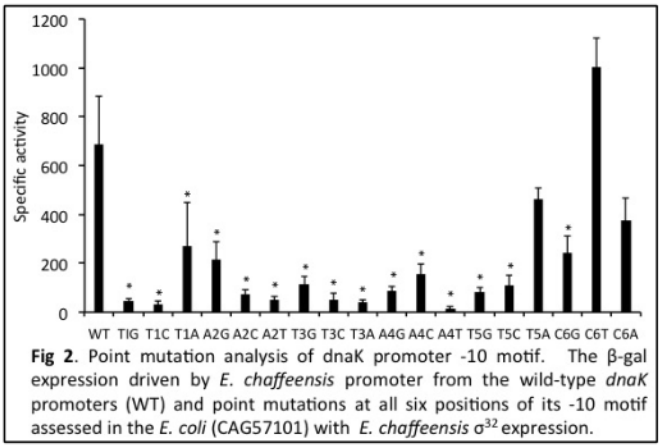

Fig 2. Point mutation analysis of dnaK promoter -10 motif. The β-gal expression driven by *E. chaffeensis* promoter from the wild-type *dnaK* promoters (WT) and point mutations at all six positions of its -10 motif assessed in the *E. coli* (CAG57101) with *E. chaffeensis* σ^{32} expression.

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A

Name	-35	Spacer	-10 +1
WT	TTGTAA	TCTTATGATTTGGTTAT	TATATCTGTGATTA
CP	TTGTAA	AGAATACTAAACCAATA	TATATCTGTGATTA
GC	TTGTAA	TCCGAGGGTCCGGTTAT	TATATCTGTGATTA
20bp	TTGTAA	TCTTATGATT ata TGGTTAT	TATATCTGTGATTA
19bp	TTGTAA	TCTTATGATT aa TGGTTAT	TATATCTGTGATTA
18bp	TTGTAA	TCTTATGATT a TGGTTAT	TATATCTGTGATTA
16bp	TTGTAA	TCTTATGATT-GGTTAT	TATATCTGTGATTA
15bp	TTGTAA	TCTTATGAT--GGTTAT	TATATCTGTGATTA
14bp	TTGTAA	TCTTATGA---GGTTAT	TATATCTGTGATTA
0bp	TTGTAA	deleted	TATATCTGTGATTA

B

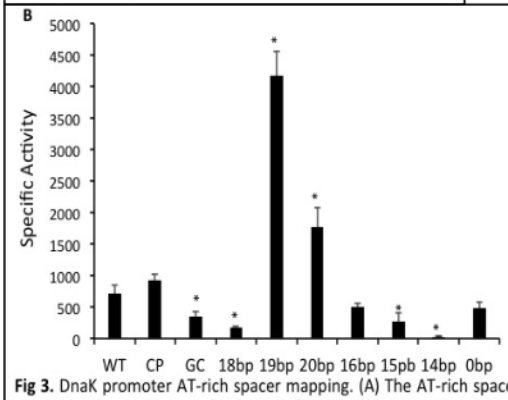

Fig 3. DnaK promoter AT-rich spacer mapping. (A) The AT-rich spacer sequence for wild-type (WT) and for modified constructs are; complementary DNA (CP), GC sequence spacer (GC), increased spacer to 18, 19 or 20 bp or decreased spacer to 16, 15 or 14 bp or deleted spacer (0 bp). (B) The β-galactosidase activity assessed for WT and all spacers in *E. coli* (CAG57101) with *E. chaffeensis* σ^{32} expression.

major conclusions from the study are that; 1) the high A+T content in spacer sequence and 2) the spacer length play a major role in altering the promoter activity. We then mapped critical determinants of the σ^{32} protein interacting with the dnaK promoter; regions 1.1 and 1.2 of σ^{32} were identified as critical for transcription initiation (not shown). This is the first detailed mapping of alternate sigma factor (σ^{32}) in *E. chaffeensis*. Together, our studies suggest that the -35 motif was important for the transcription by both σ^{32} and σ^{70}, while -10 motif is required only for σ^{32} function. *These findings are n*ow compiled for publication. [The data were presented recently at the 29th ASR meeting, June 16-19, 2018].

Zhang et al. 2013: Molecular chaperones, such as ClpB, play a critical role in stress response regulation in bacteria. We investigated how *E. chaffeensis* regulates stress response during its growth in host cells. While this research was not proposed as part of the prior R01, we reasoned that extending studies on stress response regulation is important to gain insights about *Ehrlichia* pathogenesis. Little is known about how *E. chaffeensis* overcomes host cell-induced stress. ClpB in bacteria reactivates aggregated proteins in cooperation with the co-chaperones; DnaK, DnaJ and GrpE. We performed the first biochemical characterization of ClpB from *E. chaffeensis* and demonstrated that it similarly reactivates aggregated proteins. *E. chaffeensis* ClpB mRNA (EhClpB) upregulates during its replicative stage in macrophages. We also showed that EhClpB undergoes ATP-dependent oligomerization, catalyzes ATP hydrolysis and efficiently reactivates aggregated proteins. High ClpB expression in *E. chaffeensis* in reticulate cells also suggests that it is required in reactivating aggregated proteins produced during its replication due to host-induced stress.

Kuczynska-Wisnik et al. 2017: We then investigated the protein aggregation in *E. chaffeensis* during its replication in macrophages. The *E. chaffeensis* aggregated protein fraction increases during the first 48 h post infection. Further, a ClpB inhibitor [0.5 mM guanidinium chloride (GuHCl)] causes a significant *E. chaffeensis* growth inhibition (~60%) under conditions where host cell replication is unaffected. The size of aggregated protein fraction also increases significantly with the addition of GuHCl to culture media, which also causes a preferential buildup of ClpB with aggregated proteins. Together, the data suggest that *E. chaffeensis* in the stressful host cell environment results in increased protein aggregation, which is exacerbated when ClpB is inhibited. This is the first study assessing how *E. chaffeensis* overcomes host stress. We will continue studying stress regulation as a separate research direction, independent from the current renewal application.

Specific Aim 2: Evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth. We proposed to characterize three mutant strains of *E. chaffeensis* with mutations causing loss of gene activity. We reported the three mutants earlier, which were generated by transposon mutagenesis [37]. Insertion mutations within the ECH_0230, ECH_0379 and ECH_0660 genes caused attenuation of *E. chaffeensis* in vertebrate hosts. Proposed goals of this aim were to map the gene expression profiles and to assess the value of the mutants in conferring protection against infection challenge. We accomplished the goals and published **five** manuscripts.

Nair et al., 2015: Previously, we reported that ECH_0379 codes for an antiporter protein, while ECH_0660 codes for a phage like structure protein, and ECH_0230 represents another membrane protein gene [37]. We first assessed the mutants with insertions in ECH_0379 and ECH_0660 as vaccine candidates in deer and dogs. Infection with the ECH_0379 mutant and challenge with wild-type *E. chaffeensis* one month following the mutant inoculation resulted in reducing rickettsemia compared to the wild-type infection in both deer and dogs. The ECH_0660 mutant infection cleared rapidly from the bloodstream. The wild-type infection challenge 30 days after ECH_0660 mutant inoculation also caused the pathogen's rapid clearance. This is the first study demonstrating that an attenuated mutant confers protection against wild-type *E. chaffeensis* challenge.

McGill et al., 2016: We extended the study to investigate if ECH_0660 mutant also confers protection against tick-transmission challenge. Indeed, vaccination with the mutant protects dogs from *E. chaffeensis* tick transmission. We also described cellular and humoral immune responses induced by ECH_0660 mutant

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vaccination and following *E. chaffeensis* infection. Both ECH_0660 mutant vaccination and wild-type *E. chaffeensis* infection led to very similar host responses; development of high antibody titers and Th1 response. We also described IL-17 production by peripheral blood leukocytes from Ech_0660 mutant vaccinated and wild-type infection control animals, suggesting a previously unrecognized role for IL-17 and Th17 cell response to *Ehrlichia* species. These studies serve as important critical first steps in defining the role of the immune system in vaccine-induced protection from *E. chaffeensis* infection and that ECH_0660 gene mutant is a suitable vaccine candidate against *E. chaffeensis* infections from blood transfusions or tick-transmissions.

McGill et al., 2018: We then investigated if the ECH_0230 gene mutant also promotes protection by developing sustained cellular or humoral immunity similar to ECH_0660 mutant. The Ech_0230 mutant neither promoted a long-lived immune response, nor did it confer protection against wild-type *E. chaffeensis* challenge. However, we discovered that a population of mature $CD4^+$ and $CD8^+$ double-positive (DP) T cells in the infected hosts expand significantly. Little is known about the function of these T cells, particularly in the context of an infectious disease. Using *in vitro* antigen recall assays, we demonstrated that the DP T cells undergo clonal expansion and produce IFNγ and IL-17. Together, the data revealed for the first time that DP T cells also accumulate during *E. chaffeensis* infection as a host response to tick-borne rickettsial infections.

Kondithimmanahalli and Ganta, 2018: In this study, we assessed the impact of three mutations on the transcriptome using RNA deep-sequencing technology. Mutation within an antiporter gene (ECH_0379) resulted in drastic down regulation of many transcribed genes leading to metabolic depression. Mutation downstream to coding sequence of ECH_0490 gene (with no impact to its gene expression) also caused major transcriptome changes, which included upregulation of various stress response regulatory genes. These changes may have helped the mutant to survive well in vertebrate and tick hosts [37]. Mutation within ECH_0660 gene resulted in fewer transcriptional changes, thus keeping the integrity of its transcriptome very similar to the wild-type *E. chaffeensis*. The transcriptome data correlate well to the proteome (Unpublished manuscript in review) and suggest that the ECH_0660 mutant, while losing its ability to persist in a host, offers the best protection against infection challenge [43, 44] because of its extensive transcriptome/proteome conservation similar to wild-type.

Nair et al., 2014: We investigated if deer (reservoir host) or dog (incidental host) receiving infection macrophage- or tick cell-grown *E. chaffeensis* or by tick transmission have different outcomes. Both animals develop persistent rickettsemia; while infection is detected more frequently in the blood for macrophage culture-derived inoculum as compared to tick cell inoculum or tick transmission. Tick cell-inoculum and tick transmission also caused a drop in tick infection acquisition rate compared to macrophage-grown inoculum. Independent of deer or dogs, IgG response was higher against macrophage-derived *E. chaffeensis* antigens for macrophage-inoculum compared to tick cell-derived *Ehrlichia* antigens. Likewise, animals infected with tick cell-derived inoculum or tick transmission of *E. chaffeensis* caused a higher antibody response to tick cell-derived bacterial antigens compared to macrophage-cultured antigens. The data demonstrate that the host cell-specific *E. chaffeensis* protein expression influences rickettsemia levels, humoral responses and the pathogen acquisition by ticks. The study also establishes that tick cell-derived inoculum is similar to tick transmission with reduced rickettsemia, IgG response and tick acquisition of *E. chaffeensis*. The data have important implications in understanding the disease progression resulting from a blood transfusion infection versus a tick transmission.

Specific Aim 3: Perform mutational analysis and *in vivo* screening to identify genes essential for the *E. chaffeensis* pathogenesis. We proposed to generate additional *E. chaffeensis* mutants and screen them *in vivo*. We dedicated considerable effort in developing stable targeted mutagenesis methods, while also generating a random mutagenesis library. We also worked in developing an axenic culture method to grow *E. chaffeensis* to facilitate rapid isolation of mutants. We published **four** manuscripts describing the data.

Wang et al. 2017: Targeted mutagenesis of *Ehrlichia* species and in other related rickettsial pathogens remained the most sought-after and a major challenge [45]. We reported earlier that stable mutations could be documented at several genomic locations by random mutagenesis [37]. We reasoned that targeted mutagenesis requires considerable optimization. We then carefully designed experiments to develop robust targeted mutagenesis methods, initially by targeting genomic regions where we could mutate by random mutagenesis, as a way of logically establishing targeted mutagenesis methods. We succeeded in creating several targeted mutations by allelic exchange. The presence of targeted mutations was confirmed by multiple ways; PCR, sequence and Southern blot analysis. We further developed protocols in reversing a mutation; a method valuable in testing molecular Koch's postulates. Targeted mutagenesis is now extended to several

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other genomic targets. The methods will be valuable in extending studies in other related *Ehrlichia* and *Anaplasma* species. *We will utilize this method to support parts of specific aims 1 and 2.*

Jaworski et al., 2017: To characterize *E. chaffeensis* mutants in ticks, we have developed a tick transmission model, where we eliminated the entire tick acquisition stage. We allowed the engorged *Amblyomma americanum* nymphs (fed on naïve sheep) to acquire infection by needle inoculation of cultured bacteria. The nymphs were then allowed to molt to adults. Infection rates were assessed in the adult ticks by PCR and used to infect naive animals. This method was also used for screening mutants in defining the value of pathogen genes for mutants' growth in ticks. We have also tested the ability of needle-infected ticks to transmit wild-type or mutant *E. chaffeensis* organisms to deer and dogs. Infection progression and IgG responses in deer and dogs were similar for ticks acquiring infection by our needle infection method or natural blood feeding method. The needle infection method simplifies the process of generating infected ticks. The method is useful to screen mutants to define genes critical for tick and vertebrate hosts, where interference of other pathogens likely acquired during tick feeding could be avoided. *We will use this method in support of some goals of aim 3.*

Table 1. *E. chaffeensis* Transposon mutant library with mutations sites defined (red text; insertions within an ORF)

	Locus	site	Gene product name
1	ECH_0039 ORF	34759	120 kDa immunodominant surface protein
2	ECH_0083 0084	74911	hypothetical protein ECH_0083; hypothetical protein ECH_0084
3	ECH_0104 ORF	90734	hypothetical protein ECH_0104
4	ECH_0106 0107	94117	hypothetical protein ECH_0106; hypothetical protein ECH_0107
5	ECH_0113 ORF	99608	hypothetical protein ECH_0113
6	ECH_0124 0125	113250	citrate synthase I & glutamate--cysteine ligase
7	ECH_0149 0150	141187	pyruvate dehydrogenase subunit beta; hypothetical protein ECH_015
8	ECH_0187 ORF	176793	hypothetical protein ECH_0187
9	ECH_0242 ORF	226758	hypothetical protein ECH_0242
10	ECH_0251 ORF	236424	hypothetical protein ECH_0251
11	ECH_0282 0283	264190	hypothetical protein ECH_0282; hypothetical protein ECH_0283
12	ECH_0329 ORF	317141	hypothetical protein ECH_0329
13	ECH_0340 0341	331292	glycerol-3-phosphate dehydrogenase (NAD(P)+); phage integrase family site specific recombinase
14	ECH_0362 0363	352054	putative phosphoribosylformylglycinamidine synthase I; DNA repair protein RadC
15	ECH_0368 ORF	360362	dioxygenase family protein
16	ECH_0372 0373	364550	hypothetical protein ECH_0372; dihydroorotase, multifunctional complex type
17	ECH_0445 ORF	423364	queuine tRNA-ribosyltransferase
18	ECH_0475 ORF	454669	signal recognition particle protein
19	ECH_0525 ORF	525880	hypothetical protein ECH_0525
20	ECH_0537 0538	539098	arginyl-tRNA synthetase; isoleucyl-tRNA synthetase
21	ECH_0561 ORF	566002	AcrB/AcrD/AcrF family protein
22	ECH_0579 0580	589238	type IV secretion system protein VirB8; hypothetical protein ECH_0580
23	ECH_0579 0580	589575	type IV secretion system protein VirB8; hypothetical protein ECH_0580
24	ECH_0592 ORF	598900	coproporphyrinogen III oxidase, aerobic, truncation
25	ECH_0593 0594	600196	hypothetical protein ECH_0593; acetylglutamate kinase
26	ECH_0600 ORF	606371	hypothetical protein ECH_0600
27	ECH_0605 0606	611987	glutamyl-tRNA synthetase; hypothetical protein ECH_0606
28	ECH_0614 ORF	619640	hypothetical protein ECH_0614
29	ECH_0622 ORF	630363	tRNA pseudouridine synthase A
30	ECH_0655 ORF	671099	RNA polymerase sigma-32 factor
31	ECH_0657 0658	671821	tRNA-Ser; hypothetical protein ECH_0658
32	ECH_0665 ORF	676091	phage uncharacterized protein
33	ECH_0666 ORF	676872	adenosylmethionine-8-amino-7-oxononanoate aminotransferase
34	ECH_0666 ORF	678007	adenosylmethionine-8-amino-7-oxononanoate aminotransferase
35	ECH_0669 ORF	683268	3-oxoacyl-(acyl-carrier-protein) reductase
36	ECH_0699 0670	708076	hypothetical protein ECH_0699; hypothetical protein ECH_0700
37	ECH_0705 0706	716170	peptide chain release factor 2, programmed frameshift; hypothetical protein ECH_0706
38	ECH_0750 0751	756968	DNA topoisomerase I; YjeF family protein
39	ECH_0760 0761	768120	RNA polymerase sigma factor RpoD; DNA primase
40	ECH_0769 0770	779062	exopolysaccharide synthesis protein; hypothetical protein ECH_0770
41	ECH_0837 ORF	854867	tRNA-i(6)A37 modification enzyme MiaB
42	ECH_0843 ORF	861495	recombination protein RecR
43	ECH_0866 ORF	888668	hypothetical protein ECH_0866
44	ECH_0878 ORF	898815	hypothetical protein ECH_0878
45	ECH_0930 0931	953314	putative bolA protein; pyridoxamine 5'-phosphate oxidase
46	ECH_0945 ORF	964068	hypothetical protein ECH_0945
47	ECH_0995 0996	1020175	hypothetical protein ECH_0995; ATP-dependent protease peptidase subunit
48	ECH_1008 1009	1034973	preprotein translocase, YajC subunit; DNA polymerase III, beta subunit
49	ECH_1038 ORF	1065224	hypothetical protein ECH_1038
50	ECH_1044 1045	1076550	hypothetical protein ECH_1044; hypothetical protein ECH_1045
51	ECH_1065 1066	1094096	2-oxoglutarate dehydrogenase; hexapeptide transferase family protein
52	ECH_1067 ORF	1095813	D-alanyl-D-alanine carboxypeptidase family protein
53	ECH_1081 1082	1109518	SURF1 family protein; hypothetical protein ECH_1082
54	ECH_1110 ORF	1134587	dethiobiotin synthetase
55	ECH_1127 ORF	1150113	major outer membrane protein OMP-1V
56	ECH_1144 ORF	1165318	major outer membrane protein P28-1
57	ECH_1148 1149	1169030	hypothetical protein ECH_1148; preprotein translocase subunit SecA

Cheng et al. 2015: We studied the impact of several mutations in *E. chaffeensis* on the transcription of genes near insertion sites (polar mutation effects). We demonstrated polar effects in four of the five mutations assessed in altering gene expression from the neighboring genes. Polar effects were observed independent of a mutation being present within a gene or in a non-coding region. We further investigated how the mutations impact *in vivo* growth in vertebrate and tick hosts using our tick infection model (described above). We reported differential requirements of *E. chaffeensis* genes for vertebrate and tick hosts.

Eedunuri et al., 2018: The ability to grow *E. chaffeensis* and related pathogens of the genera *Ehrlichia* and *Anaplasma* under axenic conditions will be a major advancement (also another major sought-after goal for the rickettsial research field). Although not part of the previous R01, we reasoned that it will be valuable to clonally purify mutants, and in conducting detailed biochemical and genetic studies. We initiated research in developing an axenic medium for *E. chaffeensis*. *In vitro* cultured *E. chaffeensis* organisms were fractionated into dense-cored cell (DC) and reticulate cells (RC) and incubated in the axenic medium having amino acids, nucleotides, and different energy sources. We observed bacterial protein and DNA synthesis primarily in RCs, but not in DCs. Additional modifications are required to improve conditions that support enhanced bacterial replication and transforming RCs to DCs. We will continue this investigation as a separate project.

Transposon librar Unpublished *unpublished data):* We have extended our previously established Himar 1 transposon random mutagenesis to generate a large pool of mutants. (This work was done in collaboration

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with Dr. Ulrike Munderloh, University of Minnesota.) We obtained 65 mutants (Fig. 4; Table 1). All the mutants grow well *in vitro* tick cells. Insertion sites of 57 mutants were mapped; of these, 31 had mutations in gene coding regions and 26 were within the inter-genic spacers. Several novel mutations included mutations in two p28 Omp genes (ECH_1127 and ECH_1144) encoding for Omp-1V and p28-Omp 1V, respectively. Mutations in ECH_0039 encoding for 120 kDa surface protein gene, a signal recognition particle protein gene (ECH_0475), RNA polymerase sigma factors (σ^{32} and σ^{70}) genes (ECH_0655 and ECH_0760, respectively), and in four predicted T4SS effector protein genes (ECH_0187, ECH_0878, ECH_0945 and ECH_1038) are also present. *This mutant library will serve as the preliminary data in defining genes important for E. chaffeensis in vivo growth (Specific aim 3).*

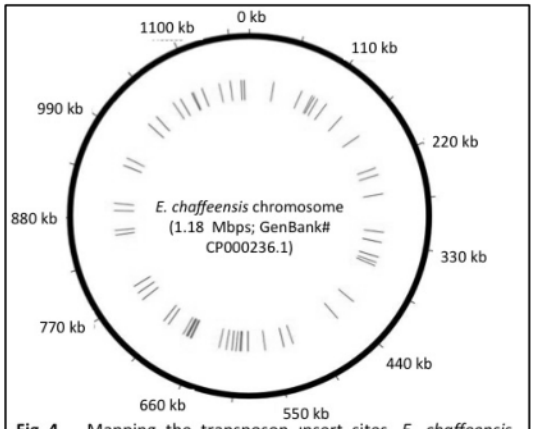

Fig 4. Mapping the transposon insert sites. *E. chaffeensis* chromosomal map showing all transposon insert sites identified in our screen. The red line refers to inserts mapped within coding sequences (31 mutants) and the blue line refers to inserts at intergenic region (26 mutants).

ADDITIONAL PRELIMINARY DATA IN SUPPORT OF THE PROPOSED SPECIFIC AIMS:

Specific aim 1: Unpublished *manuscript in progress):*

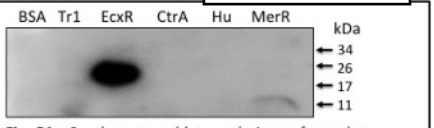

Fig 5A. Southwestern blot analysis performed to assess DNA-protein interactions of Tr1, EcxR, CtrA, HU and MerR with 7 *E. chaffeensis* gene promoters (presented data only for p28-Omp19 gene promoter).

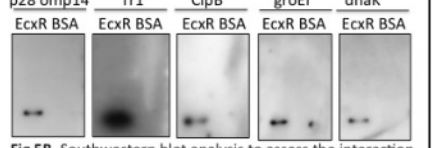

Fig 5B. Southwestern blot analysis to assess the interaction of EcxR with the 5 listed *E. chaffeensis* gene promoters.

Sequence-specific DBPs (also known as transcription regulators) control transcription of a gene by specific promoter-protein interactions in both Gram-negative and Gram-positive bacteria [46-48]. We hypothesized that DBPs in *E. chaffeensis* similarly regulate gene expression under different host environments and promote the pathogen's switch from DC to RC form and vice versa. Although some progress has been made in studying DBPs in *E. chaffeensis* [49, 50], our knowledge remains vastly limited. Likewise, it is entirely unclear how gene regulation is accomplished during progression from DC to RC and then to

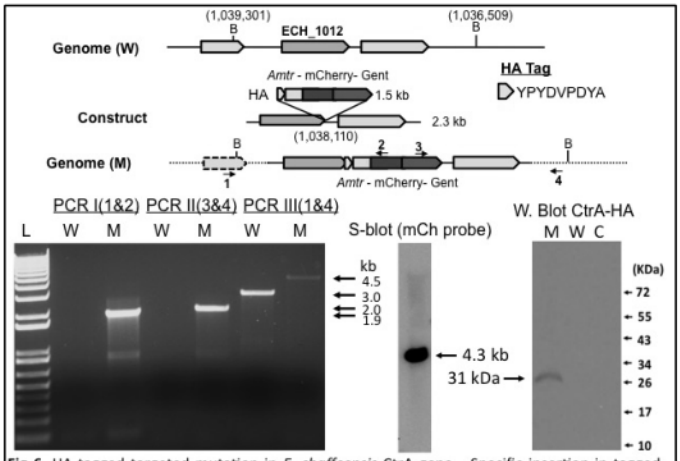

Fig 6. HA tagged targeted mutation in *E. chaffeensis* CtrA gene. Specific insertion in tagged mutation was confirmed by three different PCR assays and by Southern blot analysis. The expression of HA-tagged CtrA protein was confirmed in the mutant by Western blot analysis with the HA-specific antibody.

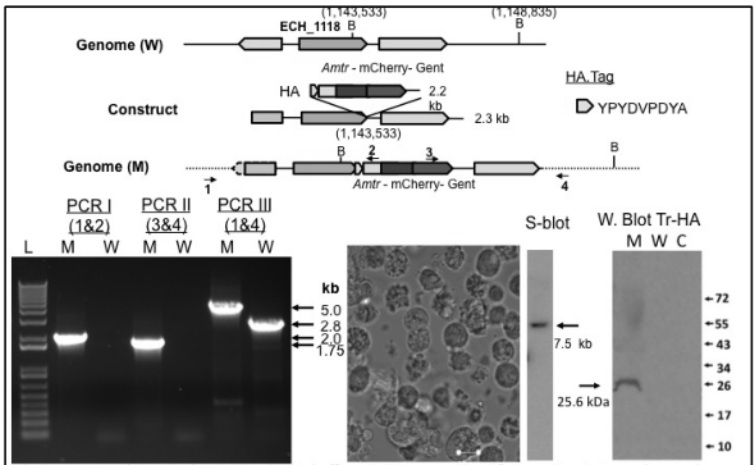

Fig 7. HA tagged targeted mutation in *E. chaffeensis* Tr1 gene. Specific insertion in tagged mutation was verified by three different PCR assays and Southern blot analysis. The expression of HA-tagged Tr1 protein was verified by Western blot analysis in bacteria expressing mCherry with a HA-specific antibody.

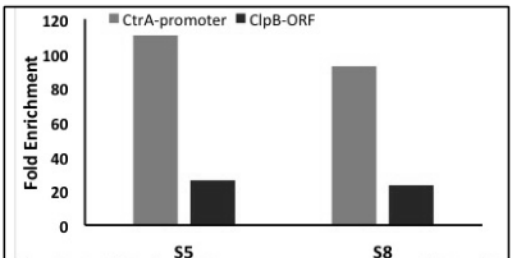

Fig 8. CtrA binding to its promoter assessed by ChIP pull down using HA antibody in CtrA-HA tagged *E. chaffeensis*. qPCR targeting to CtrA gene promoter resulted in 93-100 fold accumulation (two independent experiments), compared to background amplicons generated using non-specific antibody or for a ClpB protein coding sequence (ClpB-ORF) (negative controls).

DC within a phagosome and for subsequent release into extracellular milieu. Contributions of DBPs in regulating gene expression in different host cell conditions (macrophage and tick cell) are also not clear. We initiated investigations to assess functions of the five predicted *E. chaffeensis* DBPs [CtrA (ECH_1012), EcxR (ECH_0795), HU (ECH_0804), MerR (ECH_0163) and Tr1 (ECH_1118)] in influencing the promoter activities of 7 randomly selected genes. They included three stress response genes (*clpB*, *dnaK* and *groES*), two outer membrane protein genes (*p28-Omp 14*, *p28-Omp 19*) and two DBP genes (*hup* and *tr1*). RNA analysis by RT-PCR confirmed that all five genes are transcriptionally active in the pathogen cultured in macrophages. Recombinant proteins for the five DBPs were prepared and used to assess if they interact with the gene promoters

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by Southwestern blot analysis (Fig. 5A). Specific interactions were observed only with EcxR and for all gene promoters tested (Fig. 5B). Subsequently, electrophoretic mobility shift analysis (EMSAs) confirmed EcxR binding to all gene promoters. Promoter specific interactions were confirmed by using cold competitors and by confirming that EcxR does not bind to protein coding sequences. We also confirmed the specific EcxR binding with *tr1, p28-Omp 14* and *19* gene promoters by a yeast one-hybrid assay. We then investigated the impact of EcxR binding on the *clpB*, *dnaK*, *groES p28-Omp 14* and *19* promoter activities, which suggested that EcxR binding caused 20-60% transcription enhancement. Together, these experiments reveal that EcxR is an active global gene regulator, similar to prior studies [50].

Our newly developed targeted mutagenesis method [38] was utilized in creating insertion tags valuable in defining DNA-protein interactions of all five predicted DBPs. We have engineered mutation constructs to introduce a human influenza hemagglutinin (HA) tag at the C-terminus of the proteins. We have created two tagged mutations, Tr1 and CtrA (Figs. 6 and 7), while other work is in progress. The presence of HA tag sequences was confirmed by PCR and Southern blot analysis. Furthermore, HA tag expression as part of CtrA and Tr1 proteins was confirmed by Western blot analysis with a HA antibody (Figs. 6 and 7). We then performed chromatin immunoprecipitation (ChIP) assay to identify CtrA binding DNA segments. As previously reported that CtrA binds to its own promoter [49], we initially assessed if the HA antibody similarly pulls down the CtrA promoter. Indeed, we observed specific accumulation of CtrA gene segments (93-110 fold) with HA-specific antisera (Fig. 8). Similarly, the ChIP assay identified several novel genes, which will be investigated further as part of this competing renewal application.

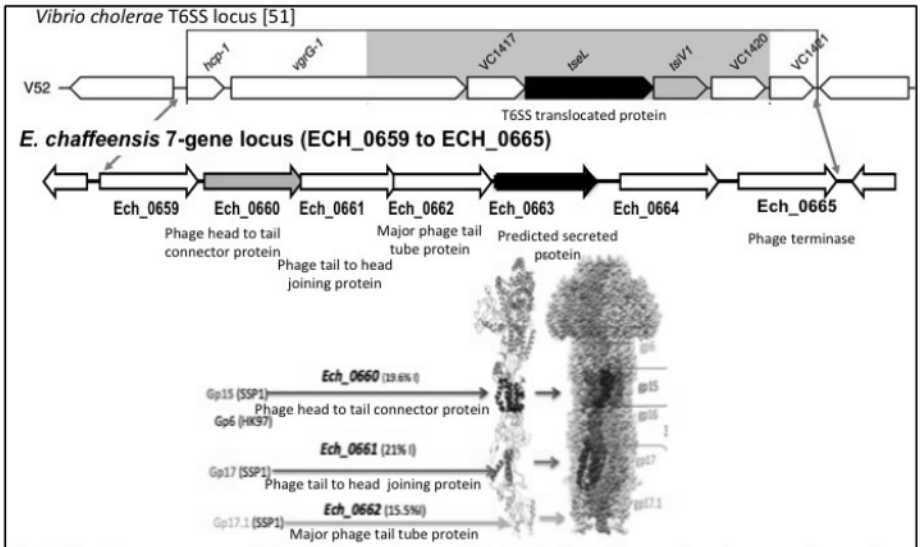

Fig 9. The 7-gene segment (ECH_0659 to ECH_0665) in *E. chaffeensis* is predicted to contain proteins having high homology to bacterial phage proteins (four genes). Its genomic organization has a structural similarity to the T6SS in *Vibrio cholerae*. Our preliminary data suggest that ECH_0663 protein from this locus is secreted into nucleus of infected macrophages (Fig 10). ECH_0663 secretion was similarly observed when assessed as a recombinant protein expression in *E. coli* (Fig 11).

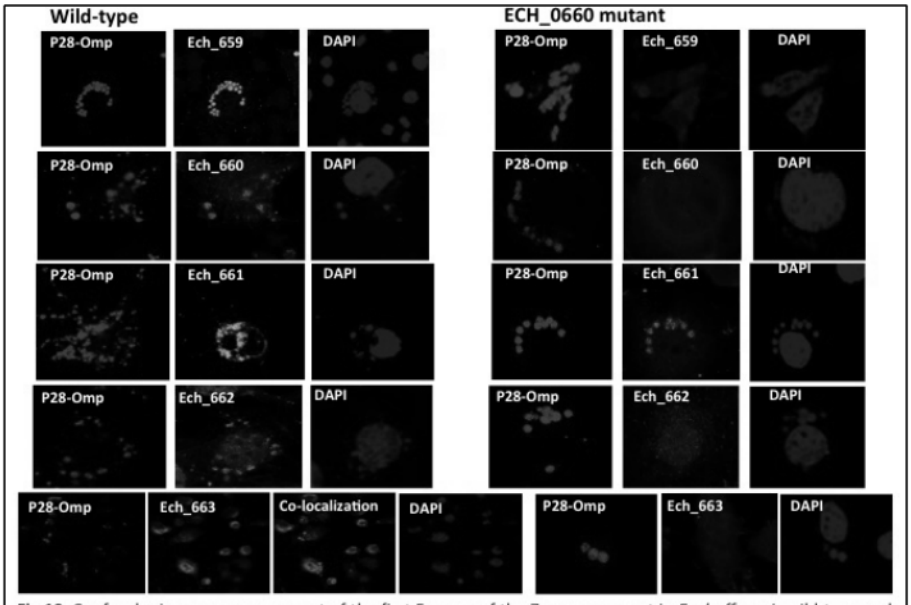

Fig 10. Confocal microscopy assessment of the first 5 genes of the 7-gene segment in *E. chaffeensis* wild-type and ECH_0660 mutant organisms cultured in macrophage cell line (DH82). Polyclonal sera were raised in rabbits for the proteins and used. All 5 proteins were expressed in wild-type and absent in the mutant, with the exception of ECH_0661. In wild-type, all five proteins were localized in morulae similar to the p28 Omp (assessed with a Ec 18.1 mAb; [32]), except ECH_0663 which stained the host cell nucleus (co-localization data shown).

Specific aim 2: We reported that the functional ECH_0660 gene is essential for *E. chaffeensis in vivo* growth [37, 43, 44]. ECH_0660 gene encodes a phage-like structure protein. Similarly, bioinformatics analysis revealed that ECH_0661, ECH_0662, and ECH_0665 genes code for phage structure proteins (Fig. 9). Further, the gene organization of the ECH_0659 to ECH_0665 (the 7-gene segment) resembled *Vibrio cholerae* Type 6 Secretion System locus (T6SS) [51]. Moreover, we observed that the G+C content of the 7-gene locus is 25%, which is 8% less than the ~G+C content of the entire *E. chaffeensis* genome (33%). Together, these data suggest that the 7-gene segment is a relatively new integration in *E. chaffeensis* genome, and possibly belonging to a bacteriophage. Phage-associated protein complexes share a common evolutionary link to T6SS [52]. T6SSs are described from a wide range of Gram-negative bacteria and aid in transporting bacterial proteins/DNA/metabolites/ions across three membranes (bacterial and eukaryotic) into the host cell cytoplasm

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or into the bacteria. T6SSs are found in about one fourth of all proteobacterial genomes, including some pathogenic bacteria [53], although not yet recognized in Rickettsiales. As several phage-like structure proteins constitute an active T6SS, we reasoned that the 7-gene segment in *E. chaffeensis* may represent a similar secretory pathway likely important for its *in vivo* growth. Further to this, our bioinformatic analysis revealed that gene homologs for this segment are present in other *Ehrlichia* and *Anaplasma* species. RT-PCR analysis suggested that all genes (except ECH_0664) were transcriptionally active in infected macrophages, whereas ECH_0660 mutant had positive RNA only for ECH_0661 (not shown). We made recombinant proteins and used them to raise polyclonal sera for the first five proteins (ECH_0659-ECH_0663). Consistent with the RT-PCR data, confocal microscopy analysis (using the respective polyclonal sera) localized the expression of all five proteins in infected macrophages for wild-type, but not for the mutant for the four of the five proteins, except ECH_0661 (Fig 10). Additionally, ECH_0663 was detected in the host cell nucleus in wild-type *E. chaffeensis*, but not in the morulae. We then constructed recombinant plasmids in *E. coli* BL21 (DE3) to assess the expression from the first fives genes of the 7-gene locus, independently. Cell lysates from each of the transformed *E. coli* were analyzed for the protein expression and localization (Fig. 11). All five proteins are associated with the bacterial membrane and/or periplasmic and cytoplasmic spaces. The ECH_0663 protein also was observed in the culture media (as a secreted protein). *In toto*, the preliminary data warrant additional investigations to determine if the 7-gene locus represents a novel secretary system in *E. chaffeensis* (proposed in aim 2).

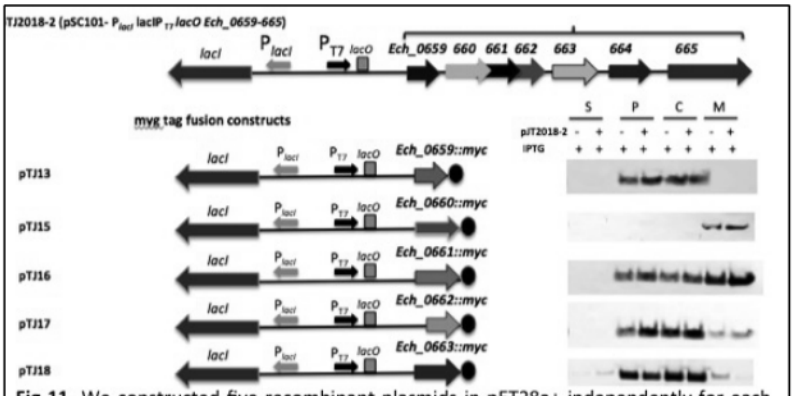

Fig 11. We constructed five recombinant plasmids in pET28a+ independently for each gene from the first 5 genes of the 7-gene locus to generate fusion proteins with a myc tag and under the control of the T7 promoter and transformed into *E. coli*, BL21 (DE3). Similarly, another plasmid containing the whole locus spanning the 7-gene locus was cloned and transformed (pTJ2018-2). Recombinant *E. coli* strains with or without pTJ2018-2 (+ or -) and with each one of the 5 gene plasmids were grown in media with IPTG to assess gene expression and localization. The bacterial cultures were harvested to isolate periplasmic (P), cytoplasmic (C) and membrane (M) fractions and subjected to Western blot analysis using a commercial anti-myc antibody. Similarly, the culture media were checked for the presence of expressed proteins. ECH_0663 was also secreted (S), as it was detected in the culture media fraction as well.

Unpublished

(manuscript in progress): Little is known about how *E. chaffeensis* and other related rickettsiales reside and replicate within an acidified phagosome (RC form). Similarly, it is unclear how DC form maintains pH homeostasis in the extracellular milieu once released from host cells. Sodium (cation): proton antiporters are integral membrane proteins present in a wide range of organisms. They exchange sodium or other monovalent cations across a plasma membrane in maintaining the cytoplasmic pH. We described a mutation within a gene predicted to encode for a Na^+/H^+ antiporter protein of *E. chaffeensis*; ECH_0379, which caused reduced bacterial presence in vertebrate hosts [37]. We reasoned that the antiporters are important for the pathogen's pH homeostasis in phagosomes. We identified 10 antiporter protein coding sequences in *E. chaffeensis* located at 6 genomic lcations. To define their function, we used a sodium sensitive *E. coli* strain (EP432) having mutations in two of its three antiporter genes to perform functional complementation assays with *Ehrlichia* genes [38]. The *E. coli* strain exhibits a growth defect in media containing NaCl and is often used to define antiporter functions of bacterial proteins [54]. With all 6 *E. chaffeensis* gene segments, using their respective gene promoters, we could demonstrate reversal of the *E. coli* sodium sensitivity at pH 5.5, while ECH_0179 and ECH_0379 genes also complemented similarly at pH 8.0 (Fig. 12). Together, these data suggest that the *E. chaffeensis* genome contains several antiporter genes

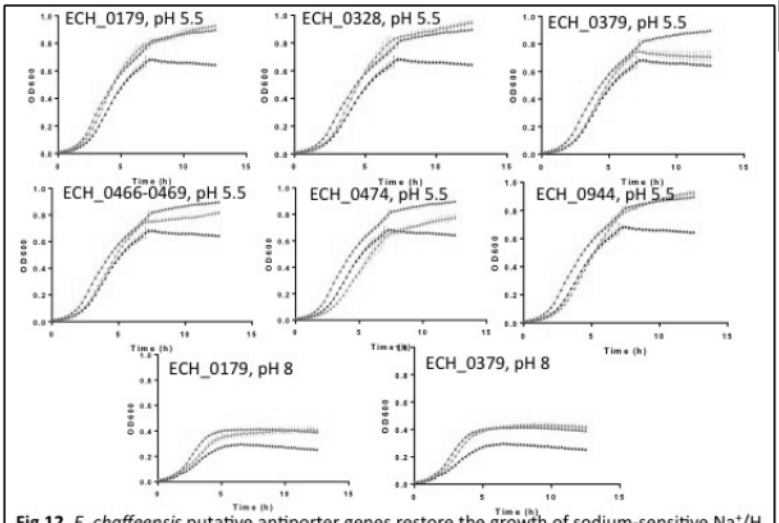

Fig 12. *E. chaffeensis* putative antiporter genes restore the growth of sodium-sensitive Na^+/H^+ antiporter deficient *E. coli* (EP432). Recombinant pBlueScript SK+ plasmids containing the predicted *Ehrlichia* antiporter genes were prepared with their respective promoter segments and transformed into EP432. The growth curves of each of the transformed *E. coli* (green) were assessed at 600 nm for 15 h in media with 200 mM NaCl at pH 5.5 or 8.0. EP432 with a non-recombinant plasmid (blue) and the plasmid containing *E. coli* antiporter gene (*NhaA*) (red) served as negative and positive controls, respectively. All genes rescued salt sensitivity at pH 5.5, while ECH_0179 and ECH_0379 also rescued at pH 8.0. (We described the assay in our recent publication [38].)

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to support its pH homeostasis within an acidified phagosome. We, therefore, proposed to further characterize these genes as part of Aim 2.

Specific aim 3: We have extended our previously established Himar 1 transposon random mutagenesis to generate 65 mutants (described near the end of progress report section above). The pool of mutants will serve as the preliminary data for the proposed experiments under Aim 3.

RESEARCH DESIGN AND METHODS: During the prior R01 project, we demonstrated our expertise in executing all the proposed project goals for the current application and included sufficient preliminary data to support the aims. The proposed three new specific aims are to continue the research in assessing the importance of differential pathogen gene expression of *E. chaffeensis* in vertebrate and tick hosts. We believe that the proposed goals are well focused in continuing our efforts in understanding pathogenesis with far-reaching goals of developing effective methods of controlling *Anaplasmataceae* pathogen diseases.

Specific aim 1: *Characterize sequence-specific DNA binding proteins (DBPs) in E. chaffeensis impacting RNA polymerase function in support of understanding the pathogen's host-specific differential gene expression.*

Premise: The project goals are justified on the premise of our published work and with the inclusion of additional preliminary data. We and other researchers earlier reported that *E. chaffeensis* gene expression is altered in a host cell specific manner [20, 27, 29, 55]. Similarly, prior studies reported that several proteins are differentially expressed in DC and RC forms of *E. chaffeensis* [56-58]. Moreover, we and other investigators reported that the pathogen gene expression varies considerably between tick- and macrophage-derived *E. chaffeensis* [20, 26]; and that the infection challenge in a host from ticks or tick cell- and macrophage-grown cultures induces different host responses [35, 36]. DBPs control transcription from a gene by their specific interactions with a gene promoter [47, 59]. DBPs play critical roles also in regulating gene expression during different environmental stresses and in inducing morphological changes. Our current knowledge is limited regarding how *Ehrlichia* and *Anaplasma* species progress from DC to RC and then RC to DC form in a phagosome and subsequent release of DCs into extracellular milieu. Similarly, a significant knowledge gap exists regarding DBPs contributions to gene expression under different host cell conditions. Goals of this aim will be addressed as **three** sub-aims.

Sub aim 1.1: Evaluation of *E. chaffeensis* lysates prepared from tick cell (ISE6) and macrophage (DH82) culture-derived RC and DC fractions.

Approach: Genes primarily transcribed by σ^{70} (*p28-Omp14* and *p28-Omp19*) and σ^{32} (*dnaK*) will be tested with *E. chaffeensis* lysates to 1) identify DBPs differentially binding to the respective gene promoters, 2) define variations resulting from host cell backgrounds, and also 3) in RC and DC forms. *P28-Omp14* and *p28-Omp19* genes were chosen as they are differentially expressed and they are primarily transcribed by σ^{70}. The *dnaK* gene was similarly selected, as it is a stress response regulatory protein gene primarily transcribed by σ^{32}. This sub-aim will allow us to gain greater understanding of gene regulation machinery in *E. chaffeensis* influenced by both predicted and undefined DBPs. *E. chaffeensis* DC and RC fractions derived from macrophage and tick cell cultures will be prepared after renografin fractionation, using methods we reported earlier [56] and *E. chaffeensis* lysates will be prepared as in [24, 60]. The lysates will then be used to assess binding of DBPs to specific regions of a gene by EMSA and DNase I foot printing assays [50, 61, 62]. DBPs bound to the respective promoter segments will be excised from polyacrylamide gels and subjected to mass spectrometry analysis to establish their identity. The assays will include appropriate controls such as cold competitor DNA segments or non-specific DNA. DNase I foot printing assays will be done to determine the exact location of sequence to which DNA binding protein interactions occur [62, 63]. The impact of DBP binding will then be assessed by measuring transcriptional variations of the genes using the *E. coli* surrogate and *in vitro* transcription systems, as in our prior publications [42, 64].

Rigor, reproducibility, and authentication plan: Three independent experiments will be performed using independently prepared lysates from macrophage and tick cell grown *E. chaffeensis*. Moreover, DNA-protein interactions will be verified using recombinant proteins prepared for some of the identified DBPs, and with various deletion constructs of promoter segments with specific mutations at certain locations.

Sub-aim 1.2: RNA and protein analysis to map variations in DBPs expressions. *Approach*: DC and RC fractions of *E. chaffeensis* cultivated in tick cells and macrophages will be purified and subjected to qRT-PCR and shotgun RNA seq analysis [65] to identify transcriptional variations for the predicted DBPs; EcxR, HU, CtrA, MerR and Tr1. Similarly, total protein lysates will be prepared from the DC and RC fractions [56] and subjected to Western blot analysis using polyclonal sera raised against all five predicted DBPs (antisera were already made in rabbits commercially using recombinant proteins). Consistent with published evidence [50],

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our preliminary studies identified EcxR as a global regulator. Similarly, HU (a histone-like binding protein) is likely another global regulator, as its homologs are known to bind numerous locations within the genome of a bacterium [66, 67]. Our working hypothesis is that the EcxR upregulates during the RC stage to activate global transcription of the pathogen, while HU expression increases during transition to DC form to downregulate global transcription. We will isolate RNA from RC and DC fractions to assess transcriptional variations in all five DBPs by qRT-PCR (our preliminary data demonstrate that all five proteins are transcribed in *E. chaffeensis*). Further, we will express all five proteins in the *E. coli* surrogate system to evaluate how they impact the transcription of several gene promoters transcribed by σ^{32} or σ^{70}. Together, the goals will help in determining how transcription varies for the five predicted DBPs in *E. chaffeensis* transition from RC to DC and vice versa, and how such variations may impact gene regulation.

Rigor, reproducibility and authentication plan: Three replicates will be used for transcriptomic analysis. The data will be independently verified by qRT-PCR and transcriptomics; we will perform time course experiments to determine changes in mRNA levels to confirm variations in the predicted DBPs. Protein analysis will similarly be verified by performing confocal analysis using polyclonal sera (which we prepared) to detect DBPs.

Sub-aim 1. 3: Targeted mutagenesis to generate fusion tags co-expressed with the five predicted DBPs for monitoring DNA-protein interactions.

Approach: In this sub aim, we will use our newly established mutagenesis method to create HA as fusion tags at the C-terminus of all five DBPs. We will then use the HA antibody in ChIP assays to pull down promoter segments interacting with the respective proteins, independently. DNA-protein interactions will then be confirmed by next-generation sequencing analysis of the genomic fragments pulled down with each tagged DBP. We already tested the feasibility of this approach by creating tagged mutations for CtrA and Tr1; while mutagenesis in other genes will be carried out similarly. As we already made two tagged mutants, we do not anticipate technical challenges, particularly since tagged mutants may not alter protein expression.

Rigor, reproducibility, and authentication plan: With the exception of targeted mutagenesis, all other experiments will be performed three independent times. Specific DNA-protein interactions will be confirmed by EMSA and Southwestern blot analysis.

Expected outcomes, potential pitfalls, and alternative strategies: We have the expertise to perform all proposed experiments. We are optimistic that we can troubleshoot any technical problems that may arise in executing the experiments. This aim will allow us to identify unique host-specific differences in gene regulation and in defining both predicted and yet undefined DBPs contributing to *E. chaffeensis* differential regulation. We will also be able to evaluate how DBPs contribute to *E. chaffeensis* transitioning between RC and DC. The goals are highly innovative in understanding how the pathogen adapts also to dual hosts.

Specific aim 2: *Characterize the functional significance of genes identified as essential for E. chaffeensis in vivo growth.* Two proposed sub-aims are to 1) define the genomic region spanning ECH_0659 to ECH_0665 genes, and to 2) evaluate sodium (cation)/proton antiporter genes in *E. chaffeensis.*

Sub-aim 2.1: *Characterize the genomic region spanning ECH_0659-ECH_0665 genes (the 7-gene segment).*

Premise: We reported that mutation in the ECH_0660 gene resulted in *E. chaffeensis* rapid clearance from both natural and incidental hosts, suggesting that this gene is important for the pathogen's *in vivo* growth. Our preliminary data also suggest that four genes of the 7-gene segment are predicted to encode phage-like proteins. The segment has a low G+C content compared to the rest of the genome. RNA and protein analysis for the first five genes of this segment suggest that four proteins are membrane/periplasmic-associated and one is translocated into host cell nucleus. Further, the 7-gene segment has a structural similarity to T6SS locus of *V. cholerae*. The preliminary data imply that it is relatively a new integration into *E. chaffeensis*, possibly from a bacteriophage genome. T6SS share a common evolutionary link with bacterial phages; T6SS is used in transport of several bacterial proteins/nutrients across bacterial and eukaryotic membranes. The preliminary data offer strong premise that the 7-gene segment encodes for a novel secretory pathway in *E. chaffeensis*, thus justifying extending studies to determine if it indeed represents a novel secretory pathway needed for the pathogen's *in vivo* growth.

Approach: We propose to perform multiple independent experiments. 1) We will map the protein expression by Western-blot analysis and confocal studies using the antisera prepared against all 7-gene proteins in wild-type *E. chaffeensis* to establish protein expression and localization of each protein. We will also fractionate nuclear, cytoplasmic, phagosome fractions of infected host cells devoid of *Ehrlichia* and similarly DC and RC forms of the pathogen, purify proteins from each fraction, and use these in localizing the proteins by Western blot analysis. We already prepared antisera against the first five proteins and made considerable headway in defining the protein localization for these proteins. Preliminary data suggest that the ECH_0663 protein is

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translocated from the pathogen into host cell nucleus, while the remaining proteins are located within the phagosome-containing bacteria and ECH_0660 mutation resulted in the loss of protein expression of four proteins, including the ECH_0663 protein. Further, our *E. coli* experiments suggest that this protein is likely a secreted protein. 1) These experiments will be extended for all 7 genes to validate the data for both the wild-type and ECH_0660 mutant. 2) We will develop targeted mutations in other genes from this locus, such as in the ECH_0659, ECH_0661 and ECH_0663 to examine how the new mutations alter protein expression from the 7-gene segment. The data will be compared with wild-type and ECH_0660 mutant. We will also determine if mutations in the ECH_0660 homologs in related *Ehrlichia* and *Anaplasma* have similar impact as observed in *E. chaffeensis in vivo.* (This work is pursued independently from the current study.) We already created a targeted mutation in the *E. canis* gene Ecaj-0381 and work in progress with an *A. phagocytophilum* homolog. 3) We will perform a fluorescent tagged mutation and an ectopic expression of ECH_0663 protein in macrophage cultures [68] to assess its specific interaction(s) with the host nucleus (within DNA and/or with proteins), similar to prior studies in *E. chaffeensis* and *A. phagocytophlium* [69-71].

Rigor, reproducibility, and authentication plan: The rigorous experimental plan safeguards validity of the data in defining the significance of the 7-gene segment for *E. chaffeensis.* We will repeat all proposed protein, DNA and RNA assessments three independent times. Targeted disruption mutations and a tagged mutation will aid in validating the conclusions drawn from this study.

Sub-aim 2.2: Evaluate sodium (cation)/proton antiporter genes in *E. chaffeensis.*

Premise: Little is known about how *E. chaffeensis* regulates its intracellular pH when replicating within acidified phagosomes. Similarly, it is unclear how the DC form differs in pH homeostasis during the extracellular milieu. Sodium (cation): proton antiporters exchange sodium or other monovalent cations across plasma membranes in regulating cytoplasmic pH. We reported that a mutation in a Na^+/H^+ antiporter protein gene of *E. chaffeensis* (ECH_0379) caused reduced bacterial presence in vertebrate hosts. *E. chaffeensis* contains several genes predicted to encode antiporter proteins. Our preliminary data, described using the functional complementation assays in a sodium sensitive *E. coli*, suggest that all predicted genes are functionally active. Thus, offering a strong premise for the proposed investigations to study the importance of multiple antiporters in support of *E. chaffeensis* pH homeostasis for its intra and extracellular survival.

Approach: We will perform RNA and protein expression analysis for all predicted antiporter genes in RC and DC forms of *E. chaffeensis* cultured in macrophage and tick cell lines for wild-type and various mutated organisms. *E. chaffeensis* RNA and protein preparations will be made as in aim 1 methods. We will characterize antiporters in wild-type *E. chaffeensis*, in ECH_0379 mutant, and by creating mutations disrupting the functions of two more genes; ECH_0328 and ECH_0474. We chose these two genes because our preliminary data suggest that RNA expression for these genes and Ech_0379 increase in macrophage-grown RC form, while RNA for ECH_0379 and ECH_0474 is absent in both RC and DC forms in tick cell-grown *E. chaffeensis*. We will also attempt to make dual mutations in combination with ECH_0379. This is to test the hypothesis that mutation in ECH_0328 or ECH_0474 may have similar impact as in the ECH_0379 mutant in altering their transcription in wild-type *E. chaffeensis*, while the double mutations may make the pathogen more sensitive to replication *in vitro* and *in vivo* in tick and canine hosts (it will be tested as part of aim 3). Protein localization will be assessed by confocal microscopy with antisera made against recombinant proteins. We already made sera against ECH_0379. Similarly, we will prepare sera in rabbits against recombinant ECH_0179, ECH_0328, and ECH_0474 proteins (using a commercial vendor).

Rigor, reproducibility, and authentication plan: A detailed experimental plan for this sub-aim includes protein and RNA analysis by confocal microscopy, Western blot analysis, qRT-PCR, and global transcriptome analysis of mutants compared to wild-type organisms cultured in macrophage and tick cells. All proposed experiments will be performed three independent times. Targeted mutation assessment in three antiporter genes will aid in defining the true significance of antiporters for *E. chaffeensis* life cycle.

Expected outcomes, potential pitfalls, and alternative strategies: This aim will further aid in defining genes essential for *E. chaffeensis* continued growth *in vivo.* We reported that the proteins made from ECH_0660 and ECH_0379 are important for bacterial *in vivo* growth, possibly to support protein transport and pH homeostasis, respectively. The goals are supported by substantial preliminary data; the investigations extend to several new *E. chaffeensis* genes/proteins by taking advantage of our newly developed mutagenesis methods. This will allow us to determine the protein expression variations, localization and the roles of phage-like structure proteins and antiporters for *E. chaffeensis in vitro* and *in vivo*. Considering the compelling preliminary data, we do not anticipate technical challenges in accomplishing the proposed goals. We anticipate gaining considerable new knowledge at the conclusion of the innovative experiments we planned in this aim.

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Specific aim 3: Mutagenesis and *in vivo* screening in defining the genomic regions critical for the bacterial pathogenesis in vertebrate and tick hosts. Two sub-aims are: 1) characterize the existing mutagenesis library, and 2) develop additional mutants to expand our library in defining genes essential for *E. chaffeensis.*

Premise: To understand the mechanisms by which bacterial pathogens overcome the immune system in causing disease, it is essential to identify pathogen proteins needed for each stage of the infection process. Identifying pathogenic proteins by *in vitro* methods is often not possible, as many contributing host factors cannot be reproduced in cell culture. We established mutagenesis and *in vivo* screening methods useful in identifying genes essential for *E. chaffeensis in vivo* growth [37, 38, 72, 73]. These methods are well suited for mapping genes important for *Ehrlichia* pathogenesis. Similar studies also aided in defining genes essential for several other pathogenic bacteria [74-77]. The primary focus of this aim is to expand the approach by screening the mutational library we developed (briefed in progress report), and additional mutant clones which will be generated as parts of all three aims.

***Sub aim 3.1** approach:* We have a library of 65 *E. chaffeensis* transposon mutants. We will create pools of up to 10 mutants each and infect three commercially reared, purebred dogs each per pool. Infections in dogs will be monitored for 8 weeks as we reported earlier [37]; blood samples will be collected twice a week and infection will be assessed by nested PCR and culture recovery. Pathogen DNA from the input pool and output pools (in dog blood) will be assessed to identify mutants excluded during *in vivo* growth. The analysis represents a total of about 50 blood samples assessed per each pool. For xenodiagnoses, nymphal *A. americanum* ticks (250 nymphs) will be allowed to acquisition feed on dogs starting from day 5-post infection. We will evaluate a minimum of 20 ticks from each animal following molt to adult stage to assess which mutants are acquired by ticks. Together, blood assessment for bacterial culture isolation and DNA by PCR, and xenodiagnosis will aid in determining which *E. chaffeensis* genomic regions are critical for *in vivo* growth. One possibility is that some mutants may grow slower in a host when injected as a pool. Thus, we will repeat the infection experiment on the mutants identified as not growing *in vivo* in the first experiment (about 30% mutants may be impacted as per our previous studies). We will use *in vivo* growth deficient mutants and make pools of 6 mutants each and repeat the infections in two animals per pool. Similarly, we will select most promising candidates (such as those with mutations in likely membrane protein genes or secreted protein genes) and perform infection experiments individually in three animals each. We successfully employed this strategy in mapping several essential *E. chaffeensis* genes. (A power analysis was performed with input from a statistician colleague in support of validating the results.) Infection assessments will be followed similarly as we performed earlier by sampling blood collected over a two-month period to examine for the presence of organisms by PCR and *in vitro* culture recovery. For *in vivo* screening in ticks, we will follow our needle inoculation infection method of generating and screening infected ticks [73]. We will create mutant pools each having 10 mutants to screen in the first round. The experiment will be repeated with new pools or individual mutants, which appear not to grow in ticks during the first round of assessment.

***Sub aim 3.2** approach:* We propose to perform additional mutagenesis experiments to expand the library of mutant clones. Based on our prior experiments, number of mutants per each transposon mutation can vary greatly from a minimum of 5 to maximum of 40. We propose performing 10 transposon experiments, which may yield between 50 to 400 (~200) new mutants in *E. chaffeensis*. These may represent mutations mostly at unique genomic locations, although some may be located within the close proximity to already defined mutations. We will assess the mutation sites by Southern blot analysis. We will then map the new mutation sites by following the protocols we already established [37]. We will also consider using whole genome sequencing, using a fee-for service commercial facility (many such facilities are available). Subsequently, we will add the mutant pools for *in vivo* screening experiments described in sub-aim 3.1. Together, we expect to identify several new mutants with mutations in genes essential for *in vivo* growth.

Expected outcomes, potential pitfalls, and alternative strategies: We have expertise in performing all the outlined experiments. Thus, we can overcome any unforeseen technical challenges during the course of experiments. Disruption of genes in the *E. chaffeensis* genome, their assessment in an incidental host and in its tick vector by screening pools and as individual mutants aids greatly in identifying many genes critical for *in vivo* growth. The outcomes will allow future investigations to study genes essential for *E. chaffeensis* and continue advancing our knowledge for rickettsial pathogenesis.

TIME FRAME: Our team of scientists, having high-level expertise in the respective experimental goals, will carry out all three aims simultaneously. We anticipate generating novel data from all three aims beginning from the years 1 and will continue through years 2 to 5.

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Peer-reviewed publications directly related to the project Specific Aims:

Aim 1) Characterize *E. chaffeensis* RNA polymerase complex in support of understanding the pathogen's host-specific differential gene expression.

1) Liu H, Von Ohlen T, Cheng C, Faburay B and **Ganta RR**. ***PLoS One*** 8(11), 2013: e81780 doi:10.1371/journal.pone. 0081780. Transcription of *Ehrlichia chaffeensis* genes is accomplished by RNA polymerase holoenzyme containing either sigma 32 or sigma 70.

2) Liu H, Jakkula LU, Von Ohlen T, **Ganta RR**. ***DNA Res.*** 2016 Jul 8. pii: dsw034. [Epub ahead of print] PMID: 27402867. Sequence determinants spanning -35 motif and AT-rich spacer region impacting *Ehrlichia chaffeensis* Sigma 70-dependent promoter activity of two differentially expressed p28 outer membrane protein genes.

3) Zhang T, Kedzierska-Mieszkowska S, Liu H, Cheng C, **Ganta RR**, Zolkiewski M. ***PLoS One.*** 2013 May 7;8(5):e62454. doi: 10.1371/journal.pone.0062454. Aggregate-reactivation activity of the molecular chaperone ClpB from *Ehrlichia chaffeensis*.

4) Kuczynska-Wisnik D, Cheng C, **Ganta RR**, Zolkiewski M. ***FEMS Microbiol Lett***. 2017 Mar 15. doi: 10.1093/femsle/fnx059. [Epub ahead of print] PMID: 28333306. Protein aggregation in *Ehrlichia chaffeensis* during infection of mammalian cells.

Aim 2) Evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for *E. chaffeensis in vivo* growth.

5) Nair AD, Cheng C, Jaworski DC, Ganta S, Sanderson MW, **Ganta RR**. ***Infect Immun.*** 2015 Jul;83(7):2827- 35. PubMed PMID: 25916990; PubMed PMCID: PMC4 4 68539. Attenuated Mutants of *Ehrlichia chaffeensis* in Inducing Protection against Wild-type Infection Challenge in the Reservoir Host and in an Incidental Host.

6) McGill JL, Nair AD, Cheng C, Rusk RA, Jaworski DC, **Ganta RR**. ***PLoS One***. 2016 Feb 3;11(2):e0148229. doi: 10.1371/journal.pone.0148229. eCollection 2016. PMID: 26841025. Vaccination with an Attenuated Mutant of *Ehrlichia chaffeensis* Induces Pathogen-Specific CD4+ T Cell Immunity and Protection from Tick-Transmitted Wild-Type Challenge in the Canine Host.

7) McGill JL, Wang Y, Ganta CK, Boorgula GDY, **Ganta RR**. ***Front Immunol.*** 2018 Jul 11;9:1585. doi: 10.3389/fimmu.2018.01585. eCollection 2018. Antigen-Specific CD4+CD8+ Double-Positive T Cells Are Increased in the Blood and Spleen During *Ehrlichia chaffeensis* Infection in the Canine Host.

8) Nair AD, Cheng C, Jaworski DC, Willard LH, Sanderson MW, **Ganta RR**. ***PLoS One***. 2014 Oct 10;9(10):e109056. doi: 10.1371/journal.pone.0109056. eCollection 2014. PubMed PMID: 25303515; PubMed Central PMCID: PMC4193820. *Ehrlichia chaffeensis* infection in the reservoir host (white-tailed deer) and in an incidental host (dog) is impacted by its prior growth in macrophage and tick cell environments.

9) Kondethimmanahalli C and **Ganta RR**. ***Sci. Rep.***, 2018 Apr 18;8(1):6162. doi: 10.1038/s41598-018-24471-3. Impact of three different mutations in *Ehrlichia chaffeensis* in altering the global gene expression patterns.

Aim 3) Perform mutational analysis and in vivo screening to identify additional genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts.

10) Wang Y, Wei L, Liu H, Cheng C and **Ganta RR**. ***Sci. Rep***. 2017 Nov 17;7(1):15801. doi: 10.1038/s41598-017-16023-y. A genetic system for targeted mutations to disrupt and restore genes in the obligate bacterium, *Ehrlichia chaffeensis*.

11) Jaworski DC, Cheng C, Nair AD, **Ganta RR**. ***Ticks Tick Borne Dis.*** 2017 Jan;8(1):60-64. doi: 10.1016/j.ttbdis.2016.09.017. Epub 2016 Sep 28.] PMID: 27729288. *Amblyomma americanum* ticks infected with *in vitro* cultured wild-type and mutants of *Ehrlichia chaffeensis* are competent to produce infection in naïve deer and dogs.

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12) Cheng C, Nair ADS, Jaworski DC, and **Ganta RR**. ***PLoS ONE*** (2015 Jul 17;10(7):e0132657. doi: 10.1371/journal.pone.0132657. eCollection 2015. PMID: 26186429). Mutations in *Ehrlichia chaffeensis* causing polar effects in gene expression and differential host specificities.

13) Eedunuri VK, Zhang Y, Cheng C, Chen L, Liu H, Omsland A, Boyle D, **Ganta RR**. ***Sci. Rep***. 2018 Jun 18;8(1):9293. doi: 10.1038/s41598-018-27574-z. Protein and DNA synthesis demonstrated in cell-free *Ehrlichia chaffeensis* organisms in axenic medium.

Other peer-reviewed publications related to the project:

14) Kim D, Jaworski DC, Cheng C, Nair ADS, **Ganta RR**, Herndon N, Brown S, and Park Y. **J. Asia-Pacific Entomol.** (Sep 2018; 21, 852-863; https://doi.org/10.1016/ j.aspen.2018.05.009). The transcriptome of the lone star tick, *Amblyomma americanum*, reveals molecular changes in response to infection with the pathogen, *Ehrlichia chaffeensis*.

15) Raghavan RK, Neises D, Goodin DG, Andresen DA, **Ganta RR**. ***PLoS ONE*** 2014 Jul 3;9(7):e100850. Bayesian spatio-temporal analysis and geospatial risk factors of human monocytic ehrlichiosis.

16) Stoffel RT, McClure JC, Butcher MM, Johnson GC, Roland W, Cheng C, Sirigireddy KR, **Ganta RR**, Boughan K, Ewing SA, Stich RW. ***Vet Microbiol.*** 2014 May 4. pii: S0378-1135(14)00217-X. doi: 10.1016/j.vetmic.2014.04.017. Experimental infection of *Rhipicephalus sanguineus* with *Ehrlichia chaffeensis*.

17) Nair AD, Cheng C, Ganta CK, Sanderson MW, Alleman AR, Munderloh UG, **Ganta RR**. ***PLoS One***. 2016 Feb 3;11(2):e0148239. doi: 10.1371/journal.pone.0148239. eCollection 2016. PMID: 26840398. Comparative Experimental Infection Study in Dogs with *Ehrlichia canis, E. chaffeensis, Anaplasma platys* and *A. phagocytophilum*.

18) Wilkerson MJ, Black KE, Lanza-Perea M, Sharma B, Gibson K, Stone DM, George A, Nair AD, **Ganta RR**. ***J Vet Diagn Invest***. 2017 Jan;29(1):109-114. doi: 10.1177/1040638716671979. Epub 2016 Nov 16. PMID: 27852813. Initial development and preliminary evaluation of a multiplex bead assay to detect antibodies to *Ehrlichia canis, Anaplasma platys,* and *Ehrlichia chaffeensis* outer membrane peptides in naturally infected dogs from Grenada, West Indies.

19) Raghavan RK, Goodin DG, Hanzlicek GA, Zolnerowich G, Dryden MW, Anderson GA, **Ganta RR**. ***Vector Borne Zoonotic Dis***. 2016 Jan 29. [Epub ahead of print] PMID: 26824880. Maximum entropy-based ecological niche model and bio-climatic determinants of lone star tick (*Amblyomma americanum*) niche.

20) Raghavan RK, Goodin DG, Dryden MW, Hroobi A, Gordon DM, Cheng C, Nair AD, Jakkula LU, Hanzlicek GA, Anderson GA, **Ganta RR**. ***Vector Borne Zoonotic Dis***. 2016 Sep;16(9):569-76. doi: 10.1089/vbz.2016.1975. Epub 2016 Jul 25. Heterogeneous associations of ecological attributes with tick-borne rickettsial pathogens in a Periurban landscape.

21) Hanzlicek GA, Raghavan RK, **Ganta RR**, Anderson GA. ***PLoS One.*** 2016 Mar 22;11(3):e0151924. doi: 10.1371/journal.pone.0151924. eCollection 2016. PMID: 27003596. Bayesian space-time patterns and climatic determinants of bovine anaplasmosis.

22) Raghavan RK, Goodin DG, Neises D, Anderson GA, and **Ganta RR**. ***PLoS ONE*****,** 2016; 4;11(3):e0150180. doi: 10.1371/journal.pone.0150180. eCollection 2016. PMID: 26942604. Hierarchical bayesian spatio–temporal analysis of climatic and socio–economic determinants of Rocky Mountain spotted fever.

23) Paddock CD, Denison AM, Dryden MW, Noden BH, Lash RR, Abdelghani SS, Evans AE, Kelly AR, Hecht JA, Karpathy SE, **Ganta RR**, Little SE. ***Ticks Tick Borne Dis.*** 2015 Apr;6(3):297-302. doi: 10.1016/j.ttbdis.2015.02.001. Epub 2015 Mar 13. PubMed PMID: 25773931. High prevalence of "Candidatus Rickettsia andeanae" and apparent exclusion of Rickettsia parkeri in adult Amblyomma maculatum (Acari: Ixodidae) from Kansas and Oklahoma.

24) Alhassan A, Liu H, McGill J, Cerezo A, Jakkula LUMR, Nair ADS, Winkley E, Olson S, Marlow D, Sahni A, Narra HP, Sahni S, Henningson J, **Ganta RR**. ***Infect. Immun.*** 2018 Nov 5. pii:

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IAI.00628-18. doi: 10.1128/IAI.00628-18. Rickettsia rickettsii whole cell antigens offer protection against Rocky Mountain spotted fever in the canine host.

Reviews/book chapters:

25) Engineering of obligate intracellular bacteria: progress, challenges and paradigms. McClure EE, Oliva Chávez AS, Shaw DK, Carlyon JA, **Ganta RR**, Noh SM, Wood DO, Bavoil PM, Brayton KA, Martinez JJ, McBride JW, Valdivia RH, Munderloh UG and Pedra JHF. ***Nat Rev Microbiol*** 15(9):544-558. doi: 10.1038/nrmicro.2017.59. Epub 2017 Jun 19

26) Mutagenesis in *Ehrlichia* and *Anaplasma* species: it's application for studies focused on understanding the pathogenesis and vaccine development. **Ganta RR**. Springer Publishers; Book title: ***Rickettsiales: Biology, Epidemiology, Molecular Biology and Vaccine Development*****,** Ed. Sunil Thomas (Ed.)

27) Identification of T cell epitopes in the murine host response to *Ehrlichia chaffeensis*. Chapes SK, Ortega MT, Cheng C, and **Ganta RR**. Springer Publishers; Book title: **Rickettsiales: *Biology, Epidemiology, Molecular Biology and Vaccine Development*****,** Ed. Sunil Thomas (Ed.)

28) Chlamydiaceae. **Ganta RR**. Book chapter in '***Veterinary Microbiology***, Third Edition' (Wiley-Blackwell Publishing, 2013). Edited by D. S. McVey, M. Kennedy and M. M. Chengappa. ISBN: 978-0-470-95949-7

29) Rickettsiaceae: *Rickettsia* and *Coxiella* species. **Ganta RR**. Book chapter in '***Veterinary Microbiology***, Third Edition' (Wiley-Blackwell Publishing, 2013). Edited by D. S. McVey, M. Kennedy and M. M. Chengappa. ISBN: 978-0-470-95949-7

30) Anaplasmataceae: *Anaplasma* species. **Ganta RR**. Book chapter in '***Veterinary Microbiology***, Third Edition' (Wiley-Blackwell Publishing, 2013). Edited by D. S. McVey, M. Kennedy and M. M. Chengappa. ISBN: 978-0-470-95949-7

31) Anaplasmataceae: *Ehrlichia* and *Neorickettsia* species. **Ganta RR**. Book chapter in '***Veterinary Microbiology***, Third Edition' (Wiley-Blackwell Publishing, 2013). Edited by D. S. McVey, M. Kennedy and M. M. Chengappa. ISBN: 978-0-470-95949-7

Manuscripts in review/preparation:

32) Unpublished

33) Unpublished

34) Unpublished

35) Unpublished

36) Unpublished

37) Unpublished

38) Unpublished

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Patent:

Ganta RR, Cheng C, Nair ADS, Jaworski D, and Ganta S. Attenuated vaccines to protect against tick-borne *Ehrlichia* species infections. Application #: WO 2016118512 A1, Jul 28, 2016.

Invited speaker presentations (2013-2018):

1) Genetic system for creating targeted mutations in tick-borne *Ehrlichia* and *Anaplasma* species to study pathogenesis and vaccine development. International Symposium in Veterinary Sciences, Federal Rural University of Rio de Janeiro, Brazil. November 6, 2018.
2) Genetic system for creating targeted mutations in *Ehrlichia chaffeensis* and its application to study pathogenesis and vaccine development. AITVM-STVM Congress meeting, September 23-28, 2018.
3) Genetic system for creating targeted mutations in *Ehrlichia* and *Anaplasma* species. 29th Meeting of the American Society for Rickettsiology, Milwaukee, Wisconsin. June 16-19, 2018.
4) Canine Infection Models and Vaccines against Ehrlichia, Anaplasma and Rickettsia species, Bohringer Ingelheim phatmaveutical company Headquarter, Lyon, FR. May 14, 2018.
5) A. Genetic System for Creating Targeted Mutations in Tick-borne Pathogens, *Ehrlichia* and *Anaplasma* species; B. CEVBD's Goals and Its Synergy with the SGU's Research Mission. St. George's University, Grenada, West Indies. March 16, 2018.
6) A genetic system for creating targeted mutations to disrupt and restore genes in *Ehrlichia chaffeensis* that is broadly applicable to other obligate bacteria. TTP9 Conference, Cairns, AU. Sep 1, 2017.
7) *Ehrlichia chaffeensis* mutagenesis: pathogenesis and vaccine development. James Cook University, Cairns, AU, Aug 29, 2017.
8) Tick-borne disease, mutagenesis, pathogenesis and vaccine development, with particular reference to *Ehrlichia chaffeensis*. Massy University, Palmerston North, NZ, Aug 23, 2017.
9) Mutagenesis exploited in developing vaccines against *Ehrlichia* species infections in dogs, Zoetis, Kalamazoo, MI. July 16, 2017.
10) *Ehrlichia chaffeensis* mutagenesis aids in defining pathogenesis and vaccine development. Madurai Kamaraj University, Madurai, India. December 29, 2016.
11) *Ehrlichia chaffeensis* mutagenesis aids in defining pathogenesis and vaccine development. Pondicherry University, Pondicherry, India. December 27, 2016.
12) Tick-borne rickettsial and other emerging diseases; challenges and opportunities. Pondicherry Veterinary Institute, Pondicherry, India. December 27, 2016.
13) Tick-borne rickettsial and other emerging diseases; challenges and opportunities. National Institute of Epidemiology, Chennai, India. December 26, 2016.
14) *Ehrlichia chaffeensis* Pathogenesis and Vaccine Development. The Beijing Institute of Microbiology and Epidemiology, China, June 30, 2016.
15) *Ehrlichia chaffeensis* Pathogenesis and Vaccine Development. Shandong University, Jinan, China, June 28, 2016.
16) *Ehrlichia chaffeensis* Pathogenesis and Vaccine Development. Yangzhou University, Yangzhou, China, June 25, 2016.
17) Metabolic and biosynthetic activity of Ehrlichia chaffeensis in a host cell-free medium. The 28th meeting of the American Society for Rickettsiology, June 11-14, 2016.
18) *Ehrlichia chaffeensis* Pathogenesis and Vaccine Development. The A. L. Bortree Lecture Series at Department of Veterinary and Biomedical Sciences, Pennsylvania State University, State College, PA. March 23, 2016.
19) Molecular approaches in understanding *Ehrlichia* pathogenesis, host-pathogen interactions and in developing vaccines. Keynote Speaker Presentation at the Conference of Research Workers on

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Animal Diseases (CRWAD) annual meeting, Chicago, IL, December 6-8, 2015.

20) Tick-borne diseases impacting the health of people, companion and agricultural animals. Animal Health Research Symposium; The Growing Risk of Vector-Borne and Zoonotic Diseases, Kansas City Area Life Sciences Institute, Kansas City, MO. August 30-31, 2015.

21) Attenuated mutants of *Ehrlichia chaffeensis* in inducing protection against wild-type infection challenge in the reservoir host and in an incidental host. The 27th meeting of the American Society for Rickettsiology, Village at Squaw Valley Olympic Valley, CA. June 20-23, 2015.

22) *Ehrlichia chaffeensis* mutagenesis to identify genes critical for persistent infection and for vaccine development. ESCCAR International congress on Rickettsia and other intracellular bacteria. Auditoire César Roux University Hospital – CHUV, Lausanne, Switzerland. June 13-16, 2015.

23) Tick-borne diseases impacting the health of people, companion and agricultural animals. Manhattan Rotary Club, April 23, 2015.

24) Animal Models and Vaccine Development against *Ehrlichia chaffeensis*. Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, July 15, 2015.

25) Ehrlichia chaffeensis mutagenesis to identify genes critical for persistent infection and vaccine. Distinguished Scientist Seminar at the University of South Alabama College of Medicine, March 26, 2015.

26) Attenuated transposon mutants of *Ehrlichia chaffeensis* induce protective host response against wild-type infection challenge. Global Summit on Emerging Science and Technologies: Impact on Environment and Human Health Special Symposia on Cancer Therapy and Environmental Toxicology. Vikramasimhapuri university, Nelluru, AP, India, August 1-3, 2014.

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PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

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VERTEBRATE ANIMALS: In this study, we will be using specific pathogen free dogs. Rabbits will also be used at a commercial site for generating antisera. Dogs will be used for experiments proposed in aims 3, while rabbits will be required for generating polyclonal sera against five different proteins. We propose to infect pools of up to 10 mutants each in three independent animals and the infection status will be assessed twice a week for two months (60 animals). Nymphal ticks (typically about 250) will be allowed to acquisition feed on animals starting from day 5 post infection. About 7 days following tick attachment, ticks will be collected by opening the chamber lids. We will evaluate ticks from each animal following the molting to adult stage to assess which mutants are acquired by ticks. Together, the assessments of blood (blood drawn twice a week for the first two weeks and then on once a week) and tick sampling will help us determine which genomic regions of E. chaffeensis that are critical for the in vivo growth in an incidental host model with important implications in extending the observations in understanding pathogenesis in people. We will repeat the infection experiment on all those mutants identified as attenuated in vivo in the previous experiment. As in the previous experiment, blood draws and tick attachment experiment will also be performed and essentially as outlined above. We will use clonally purify mutants, randomly select pools containing 5 mutants each and use them to repeat the infections in three dogs each as in the previous experiment. Total dogs to be used for this experiment will be 36. We will select 10 most promising candidate mutant clones from experiment 2 (those containing mutations in likely membrane protein or predicted secreted protein genes) and repeat infection experiments using individual mutants; three dogs per mutant clone (total 30 dogs). Similar to experiments 1 and 2, blood draws and tick attachment experiments will be performed. Infection assessments will be followed similarly as in experiments 1 and 2 by sampling blood collected over a two-month period and examine for the presence of organisms by PCR and in vitro cultivation. Total number of dogs required for accomplishing the goals will be 126. A total of 10 rabbits will also be used for preparing the polyclonal sera made against three different antigens (in support of Aim 2). The antibody preparation will be performed at a commercial vendor as per its approved protocol. Vertebrate animal care for dogs is outlined below.

Vertebrate Animals Statement:

1. The proposed research will utilize dogs as hosts for bacterial pathogenesis studies and tick acquisition/transmission studies.
2. *Ehrlichia chaffeensis* is responsible for causing human monocytic ehrlichiosis and it also causes infections in dogs (similar to humans). We have developed the dog infection challenge model, as this is an ideal host for defining various aspects, including assessing pathogenesis and vaccine development. Specific pathogen-free beagle breed dogs are readily available from commercial vendors. We will require dogs to accomplish the goals of experiments proposed under aim 3.
3. Daily care for the dogs will be managed by the Laboratory Animal Resource staff. Dogs are offered fresh water and feed ad lib and monitored by attending animal care personnel twice daily. Veterinary care which includes daily cleaning of cages and observing animals for normal behavior. The AALAC certified facility and the animal care group at Kansas State University include several attending veterinarians and trained animal care workers, all of whom are supervised by the Director of the Laboratory Animal Resources. Animal care is also available when needed from the K-State Veterinary Medical Hospital.
4. Dogs will be subjected to venipuncture, intra-venous injections of bacteria or by tick transmission experiments and that cause only momentary discomfort. Blood samples, about 5 cc per sample, will be collected twice weekly, while bacterial injections as outlined in the proposal will be done less frequently. Infected animals will be monitored for the symptoms of infection. Symptoms in dogs are generally mild with *E. chaffeensis* infections and do not result in serious outcomes. The protocol for the proposed

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experiments is already approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC).

5. Euthanasia will be performed in where applicable in accordance with the University IACUC recommendations that are consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

Similarly, rabbits will be used for preparing antisera at a commercial vendor location. The description of the rabbit use and care is included in the following documents obtained from the vendor.

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Redacted by agreement

Dear Sir or Madam:

Redacted by agreement rabbit immunization protocols and animal handling procedures are approved by NIH and USDA through Redacted by agreement and their animal facility located in Redacted by agreement Every protocol is submitted for internal review by the IACUC committee, which makes recommendations and assures that the use of animals is justified and that animals are cared for properly.

A staff of trained husbandry personnel supports the daily activities of feeding, cleaning, and routine treatments of animals. Animals are observed daily for condition and appetite by husbandry personnel, and veterinarians monitor all reporting.

Antiserum collection procedures involve only minor discomfort and anesthesia is not needed or used. Restraint of the animals is limited to the short periods of time, and the devices used for this purpose are produced commercially for this specific purpose. In the event of illness or injury a veterinarian is included in the assessment, treatment, and monitoring of the animal's condition.

The Animal Care and Use Committee (IACUC) review all protocols and procedures at least twice per year. One member of the committee is a veterinarian, three members are husbandry staff, and one member is a non-affiliated community member.

NIH (OLAW) assurance number: A3669-01; expires 03/31/2020
USDA (research license) registration number: 23-R-0089; expires 06/06/2020
PHS assurance number: A3669-01; expires 03/31/2020

Redacted by agreement products continues to maintain the highest standard for the care and treatment of animals used to produce antibodies. We continue to look for new alternatives to the use of animals, but up to this point, a substitute has not been found that provides such a critical research tool to help in understanding protein's roles in human disease.

Detailed protocol approvals by IACUC can be provided upon request.

Sincerely,

Redacted by agreement

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Information for NIH grant submissions

1. Species, strains, age, sex, numbers to be used, protocol for work

   New Zealand White Rabbits, approximately two to four month old, either sex (males will be used unless females specifically requested)

2. Justify use of animals and choice of species

   New Zealand White Rabbits are known to respond to a wide range of antigens. The use of two rabbits per antigen is justified to compensate for individual animal responses and to obtain the required volume of antisera necessary for this project.

3. Procedures for ensuring that discomfort, distress, pain and injury will be limited to that which is unavoidable in the conduct of the scientifically sound research. Describe the use of analgesic, anesthetic and tranquilizing drugs and or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain and injury.

   All technicians working with the animals are highly trained in the procedures to be performed in the production of the antibodies. Proper restraint of the animals is used during injections and blood collections to avoid discomfort, distress, pain or injury. For injections, the rabbit is held by one technician and the injection is administered by another. For blood collection, the rabbit is placed in a stainless steel restrainer to restrict the movement of the animal. Routine procedures such as injections and blood collection produce only mild, transient pain or discomfort, which do not require intervention.

4. Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. If not, present justification for not following the recommendations.

   The rabbits are exsanguinated after being anesthetized to surgical plane with a cocktail of Ketamine (0.5ml of 100mg/ml for average 6lb. Rabbit) and Xylazine (0.5ml of 20mg/ml for average 6lb. Rabbit). Exsanguination is via cardiac puncture. Cervical dislocation insures death. If the rabbit is to be euthanized without exsanguination, Beuthanasia-D is administered IV at a dose of 1ml for an average 6lb rabbit. These methods are consistent with the guidelines of the Panel on Euthanasia of the American Veterinary Medical Association.

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Vertebrate Animals

Contact PD/PI: Ganta, Roman Reddy

Obtained via FOIA by White Coat Waste Project

USDA

United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Animal Care

EXPIRATION DATE: JUNE 6, 2020

This is to certify that

COCALICO BIOLOGICALS INC

is a registered under the

CLASS R RESEARCH FACILITY

Animal Welfare Act

(7 U.S.C. 2131 et seq.)

Certificate No. 23-R-0089

Customer No. 360

[redacted] Personal Info

Deputy Administrator

Redacted by agreement

PROTOCOL NUMBER: 161027PBI90DAYRABBIT

PBI 90 Day Rabbit Standard Polyclonal Antibody Production Protocol

This protocol may be used for a variety of immunogens including but not limited to proteins, peptides (free, conjugated or MAP constructs), GST fusions, etc.

Day	Procedure
0	Prebleed/Initial inoculation
14	Boost
28	Production bleed
42	Boost
56	Production bleed/ Boost
70	Production bleed
72	Production bleed

Additional procedures such as boosts, test bleeds, production bleeds and/or the exsanguination are based on the assay results

Applicable procedural standard operating procedures/protocols include:

Species	Protocol number	Covered procedure
Rabbits	ADP-051	Master protocol
Rabbits	ASP-309, ADP-316, ASP-330	Humane handling and care
Rabbits	ADP-312	Immunizations
Rabbits	ADP-316	Quarantine procedures
Rabbits	ADP-318, ADP-319, ADP-319A	Blood collection
Rabbits	ADP-320, ADP-325, ADP-350	Veterinary care, animal health
All species	ADP-004	Pre-procedural, post procedural care
All species	ADP-500	Moribund policy
All species	ADP-501	Anesthesia of laboratory animals

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PROTOCOL NUMBER: 161027PBI90DAYRABBIT

PBI 90 Day Rabbit Standard Polyclonal Antibody Production Protocol
(continued)

Additional notes:

1. Complete Freund's Adjuvant is used only for the initial inoculation unless prior approval secured from IACUC.
2. Foot pad injections are strictly prohibited in rabbits. Foot pad injections in rodents without the use of Complete Freund's Adjuvant are approved by the IACUC following strict guidelines (see approved protocol).
3. The frequency of the bleeds is based upon the size, health, hematocrit, etc. of the host species and the requirements of the investigator.

This protocol has been approved by the Animal Care and use Committee of Redacted by agreement

Redacted by agreement

Approval date: October 27, 2016

Signature of IACUC Chairperson: Personal Info

Signature of Institutional Official:

Redacted by agreement

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RESOURCE SHARING PLAN:

The PI acknowledges that NIH considers the sharing of unique research resources developed through an NIH sponsored research an important means to enhance the value and further the advancement of the research. When resources have been developed with NIH funds and the associated research findings published or provided to the NIH, the findings will be made readily available for research purposes to qualified individuals within the scientific community.

This R01 Renewal Application does not require the following specific sharing plans:

1) Data Sharing Plan- The project does not seek $500,000 or more in direct costs in any year.

2) Sharing Model Organisms- The project does not anticipate the development of model organisms.

3) Genome Wide association Studies- The project does not seek funding for a genome-wide association study.

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Authentication of Key Biological and/or Chemical Resources

Overview:

Our project will utilize many standard laboratory reagents, which will be obtained commercially. We typically obtain molecular biology grade and cell culture grade reagents and also from the most reliable vendors to ensure the reagents are of high quality and to avoid possible experimental variations. Further, we will also keep the log of sources and in all repeated experiments, every effort will be made to use the same source or batch as much as possible. The commercial vendor details are included in publications. These details help in other investigators to be able to reproduce our results. *Ehrlichia chaffeensis* wild-type and mutant organisms which will be developed as part of the study or were made earlier, other bacterial strains and plasmids created in this project will be validated using multiple independent molecular methods; Southern blot, PCR, restriction enzyme analysis and DNA sequencing prior to archiving. The validity of reagents stored and recovered will also be confirmed by above listed one or more methods prior to reintroduction for an experiment or when sharing with a colleague. Information regarding commercial antibody lot numbers will be stored in laboratory notebooks. Antibodies generated from a commercial vendor will be validated against appropriate antigens and by performing experiments with additional control antigens to ensure the sensitivity and specificity of such reagents. Authentication of new *E. chaffeensis* mutants generated as part of this study will similarly be verified by several independent molecular methods, various microscopic and cell culture methods with the use of appropriate antibodies. We will maintain stringent authentication criteria and documentation plans by research personnel executing the experiments.

1. Cell Lines/Plasmids/Nucleic acids

We use the *E. chaffeensis* Arkansas isolate that we originally obtained from the CDC. It has been actively maintained and its integrity is frequently checked by molecular methods. It is also available at ATCC. Similarly, we will confirm the authenticity of various mutant strains of the organism, which we developed in the past and are to be developed. We typically verify the validity of a strain once every two to three months. We also keep our biological safety cabinets certified annually and similarly all lab equipment items are quality checked and maintained frequently on a regular basis. If we experience culture contaminations, we will discard all cultures and start from a frozen stocks and proceed with experiments only after confirming their validity. *E. coli* strains used for molecular studies are/will be obtained from a legitimate and reputable commercial vendor . All engineered plasmids and transformation experiments in *E. coli* strains will be confirmed by antibiotic selections, restriction enzyme analysis and by other molecular methods, such as PCR and sequencing. All nucleic acids, including plasmid DNA to be used are verified by following standard molecular methods, such as PCR, RE analysis, Southern blot and sequencing.

2. Antibodies/Recombinant proteins

Most of the antibodies to be used in the proposed studies are either generated or will be generated by us. We prepare recombinant proteins and verify their integrity on a stained polyacrylamide gel followed by performing mass-spectrometry analysis before sending to a commercial vendor (Thermo-fisher Scientific) for making polyclonal sera in rabbits. Once sera are obtained, we will check their specificity and sensitivity against the antigens we prepare and also using non-specific antigens at different concentrations of both sera and antigens.

3. Specialty Chemicals

We will not use any special chemicals in our study.

4. Vertebrate Animals

We typically purchase dogs from Covance Research Products Inc. or from Marshall BioResources. These are reputable vendors and the animals represent purpose-bred beagles. Similarly, several reputable companies sell research-grade animals (https://guide.labanimal.com/product/breed). The animals to be used for the experiments are purchased exclusively for use of our project and having no prior history of engaged in any other studies. Upon arrival, we acclimatize them at the KSU animal facility, and all dogs will be health assessed by a veterinarian. We will also perform complete blood analysis and confirm that the animals are healthy. Further, we will perform molecular and serological assessment to confirm that there was no prior exposure to tick-borne diseases.

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Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/06/2020

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI070908-11
FAIN: R01AI070908

Principal Investigator(s):
ROMAN R. GANTA, PHD

Project Title: Vector and Host Contributions to the Regulation of E. Chaffeensis Gene Expression

Paul R. Lowe
Kansas State University
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Manhattan, KS 665061103

Award e-mailed to: ksuawards@ksu.edu

Period Of Performance:
Budget Period: 07/01/2020 – 06/30/2021
Project Period: 12/15/2007 – 06/30/2024

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $536,311 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI070908. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

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Sincerely yours,

Shan Liang
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

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SECTION I – AWARD DATA – 5R01AI070908-11

Award Calculation (U.S. Dollars)

Federal Direct Costs	$352,836
Federal F&A Costs	$183,475
Approved Budget	$536,311
Total Amount of Federal Funds Obligated (Federal Share)	$536,311
TOTAL FEDERAL AWARD AMOUNT	$536,311
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$536,311

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
11	$536,311	$536,311
12	$536,311	$536,311
13	$536,311	$536,311
14	$536,311	$536,311

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1480771751A1
Document Number:	RAI070908D
PMS Account Type:	P (Subaccount)
Fiscal Year:	2020

IC	CAN	2020	2021	2022	2023
AI	8472364	$536,311	$536,311	$536,311	$536,311

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C B / **OC**: 41025 / **Released:** eRA Commons User Name 07/06/2020
Award Processed: 07/07/2020 12:01:02 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI070908-11

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI070908-11

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain

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references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI070908. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI070908-11

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

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Any noncompliant publications reported on the RPPR dated 05/14/2020 are required to be brought into compliance before 6/30/2021. Failure to comply with this term may result in actions that may affect future funding.

*****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

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Grants Management Specialist: Michael W. Fato
Email: mfato@niaid.nih.gov **Phone**: 240-669-2968

Program Official: Samuel S. Perdue
Email: sperdue@niaid.nih.gov **Phone**: 240-627-3341

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI070908-11

INSTITUTION: KANSAS STATE UNIVERSITY

Facilities and Administrative Costs	Year 11	Year 12	Year 13	Year 14
F&A Cost Rate 1	52%	52%	52%	52%
F&A Cost Base 1	$352,836	$352,836	$352,836	$352,836
F&A Costs 1	$183,475	$183,475	$183,475	$183,475

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A. COVER PAGE

Project Title: Vector and Host Contributions to the Regulation of E. Chaffeensis Gene Expression	
Grant Number: 5R01AI070908-11	**Project/Grant Period:** 12/15/2007 - 06/30/2024
Reporting Period: 07/10/2019 - 06/30/2020	**Requested Budget Period:** 07/01/2020 - 06/30/2021
Report Term Frequency: Annual	**Date Submitted:** 05/14/2020
Program Director/Principal Investigator Information: ROMAN R GANTA , PHD MS BS **Phone number:** 785-532-4612 **Email:** rganta@vet.k-state.edu	**Recipient Organization:** KANSAS STATE UNIVERSITY KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL, 1601 VATTIER STREET MANHATTAN, KS 665062504 **DUNS:** 929773554 **EIN:** 1480771751A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu	**Signing Official:** PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

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B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS: Rickettsial diseases caused by the Anaplasmataceae family pathogens of the genera Ehrlichia and Anaplasma remain a growing public health concern spanning the past three decades, and are also the second leading cause of tick-borne infections in humans throughout the USA and many parts of the world. The diseases include human monocytic and granulocytic ehrlichiosis caused by Ehrlichia chaffeensis and Ehrlichia ewingii respectively, and human granulocytic anaplasmosis resulting from Anaplasma phagocytophilum infections. Recently, another tick-borne pathogen, Ehrlichia muris subsp. eauclairensis, has been identified as causing another ehrlichial disease in people. Despite the complex cellular environments of arthropods and vertebrates having sophisticated systems of defense, these pathogens have evolved strategies to evade host clearance. For example, E. chaffeensis in macrophages and tick cells has distinct patterns of protein expression and the pathogen translocates effectors in altering host responses to support its continued survival in a host. Our prior studies demonstrated that differential expression in vertebrate and tick cells alters host responses leading to variations in vertebrate host clearance/persistence. The central hypothesis of our competing renewal application remains that E. chaffeensis differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for the pathogen's continued survival in vertebrate and tick cell environments. Specific aims of the previous funded project are to 1) characterize the E. chaffeensis RNA polymerase complex in understanding the pathogen's host-specific differential gene expression; 2) evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for the in vivo growth of E. chaffeensis; and 3) perform mutational analysis and in vivo screening to identify additional genes essential for E. chaffeensis pathogenesis in vertebrate and tick hosts. We made substantial progress in addressing all three specific aims. The progress, as evidenced in numerous peer-reviewed publications (24 peer-reviewed publications and 7 reviews/book chapters), paved the way for new directions of this renewal application. The new specific aims of this renewal application continue to address the above stated hypothesis. The proposed aims are supported with a strong premise. Furthermore, the proposed project goals are highly innovative and attempt to fill-in a significant gap of knowledge. We are the only research group investigating the detailed regulation of gene expression in Ehrlichia species and such studies may be extended in other Ehrlichia and Anaplasma species pathogens. We also lead the investigations on targeted mutagenesis studies for Anaplasmataceae pathogens, including in creating mutations disrupting and restoring a gene function. We used the methods in studies to understand pathogenesis and in studying gene regulation in Ehrlichia species. Targeted mutations to disrupt and also to functionally restore a gene will be valuable in testing molecular Koch's postulates. We have also created targeted mutations to generate bacterial fusion proteins, which are important in studies to characterize E. chaffeensis proteins in vivo. Additionally, we developed physiologically relevant animal infection models. Moreover, we standardized tick transmission methods in support of understanding the bacterial pathogenesis, host responses and vaccine development. We also extended studies in developing an attenuated vaccine to protect an incidental host from E. chaffeensis by needle and tick transmitted challenge. The in vivo infection models will also be valuable in investigating the importance of genes to the E. chaffeensis life cycle in tick and vertebrate hosts. Collectively, the extensive novel data, generated with the support of our previous R01 grant, form a strong foundation for the proposed three specific aims of this renewal application.

Specific Aims:
1)Characterize sequence-specific DNA binding proteins (DBPs) in E. chaffeensis impacting RNA polymerase function in support of understanding the pathogen's host-specific differential gene expression.
2)Characterize the functional significance of genes identified as essential for E. chaffeensis in vivo growth.
3)Mutagenesis and in vivo screening in defining genomic regions critical for the bacterial pathogenesis in vertebrate and tick hosts.

The proposed specific aims have a strong scientific premise and are a logical extension of the substantial progress we made during the previous funding cycle. The project goals are supported by the research data reported in our several peer-reviewed publications and with the inclusion of additional data. We have sufficient expertise to ensure successful execution of the project, and we included well thought-out authentication plans. All molecular tools to study gene expression were developed by us. We also have accomplished mutagenesis and in vivo screening methods to test the importance of E. chaffeensis genes in tick and vertebrate hosts. We believe that the goals of this renewal project are also critical in advancing understanding of the Ehrlichia pathogenesis. We expect that our continued progress will pave the way for similar studies in related rickettsials in controlling diseases caused by Anaplasma, Ehrlichia and Neorickettsia species.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Ganta NIH R01 Progress report final May 12 2020.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We will continue our work on all three specific aims of the proposal to further the progress on the proposed goals. We will continue our studies in evaluating the five identified DBPs, and also initiate research on sub-aims 1.1 and 1.2 with a focus to evaluate additional E. chaffeensis DBPs expressed when the pathogen is replicating in tick cell and macrophage environments during its developmental stages; reticulate cells and dense core cells. Efforts will also be under way to map variations in the DBPs assessed by RNA and protein analysis during the course of the pathogen developmental stages in tick and macrophage cell environments. We will also extend studies under specific aim 2 in defining the functional significance of genes identified as essential for E. chaffeensis in vivo growth, including ECH_0379 and ECH_0660.

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A. SPECIFIC AIMS: The project goals are to study how *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals for its survival in vertebrate and tick cell environments. Three specific aims of the project are to 1) characterize sequence-specific DNA binding proteins (DBPs) in *E. chaffeensis* impacting RNA polymerase function in support of understanding the pathogen's host-specific differential gene expression; 2) characterize the functional significance of genes identified as essential for *E. chaffeensis in vivo* growth; and 3) mutagenesis and *in vivo* screening in defining genomic regions critical for the bacterial pathogenesis in vertebrate and tick hosts.

B. STUDIES AND RESULTS:

Specific Aim 1: Characterize sequence-specific DNA binding proteins (DBPs) in *E. chaffeensis* impacting RNA polymerase function in support of understanding the pathogen's host-specific differential gene expression.

This is a very complex and challenging goal and we proposed three sub-aims. We initiated the work on the sub-aim 1.3; *Targeted mutagenesis to generate fusion tags co-expressed with the five predicted DBPs for monitoring DNA-protein interactions.* This sub-aim's goals are to investigate the five predicted DBPs of *E. chaffeensis* for their role in DNA-protein interactions in support of RNA polymerase function. Similar to other obligate bacteria, most of the RNA polymerase characterization experiments must be performed using *in vitro* systems and requires novel tools. Substantial progress was made during the previous funding cycle, which forms the strong foundation for the current investigations. Our prior studies led to the development of a novel surrogate system in *Escherichia coli* to study *E. chaffeensis* transcriptional regulation. This system, together with the *in vitro* transcription system developed earlier (as part of our previously funded portion of the grant) was used to map promoter motifs of two differentially expressed *E. chaffeensis* p28 Omp genes whose gene expression is primarily regulated by its σ_{70} (Liu *et al.* 2016). We then extended studies to another important gene coding for the *E. chaffeensis* stress response protein, DnaK that is transcribed by RNA polymerase with the sigma factor, σ_{32}. We have performed detailed mapping of the promoter regions of the gene involved in transcribing the transcript encoding for DnaK. This work was compiled as a manuscript and published in a peer-reviewed scientific journal (Liu *et al.* 2019).

Sequence-specific DBPs (also known as transcription regulators) control transcription of a gene by specific promoter-protein interactions in both Gram-negative and Gram-positive bacteria. In prokaryotes, transcriptional specificity of genes is accomplished by RNA polymerase with DNA binding proteins. The ability of *E. chaffeensis* to regulate its gene expression in tick and vertebrate hosts is currently poorly understood. Five predicted DBPs in *E. chaffeensis* include; EcxR, Tr1, CtrA, MerR and HU. EcxR has a DNA-binding winged helix-turn-helix (HTH) motif. Tr1 homologs are known to be involved in xenobiotic response with the cro/C1-type HTH DNA-binding domain. CtrA homologs have been shown to be involved in two-component signal transduction systems having a DNA binding domain at the C-terminal part. MerR, as part of the MerR family transcription regulators, mediates responses to stress including exposure to heavy metals, drugs, or oxygen radicals and regulates transcription of multidrug/metal ion transporter genes and oxidative stress regulons, as evidenced by the data generated in several bacteria. HU is predicted to be a histone-like protein, which plays a critical role in global regulation of transcription by binding DNA in bacterial genomes. We initiated studies to test the hypothesis that one or more of the five predicted *E. chaffeensis* DBPs play an important role in regulating gene expression. All five putative DBPs of *E. chaffeensis* (EcxR, CtrA, Tr1HU, and MerR) were assessed for their roles in influencing the promoter activities of 7 selected genes coding for ClpB, DnaK, GroES/L, HU, p28-Omp 14, p28-Omp 19, and Tr1. Recombinant proteins for the putative DBPs were made using a *E. coli* expression system and were used to assess interactions with the above listed 7 gene promoters. Specific interactions of all the promoters with EcxR were observed and the data were independently confirmed by Electrophoretic Mobility Shift Assay (EMSA), South-Western blot analysis and using a Yeast hybrid system. This work is compiled for publication Unpublished manuscript in progress.)

The chromatin immunoprecipitation (ChIP) assay is a powerful and versatile technique used for probing protein-DNA interactions within the natural chromatin context of a cell for identifying binding regions of a particular DBP to genome sequence(s). The ChIP-grade anti-HA (human Influenza hemagglutinin) tag

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antibody has been successfully used in various ChIP assays. By taking advantage of our newly developed targeted mutagenesis system (Wang *et al.*, 2017), we created mutation constructs to introduce HA tags at the C-terminuses of the five DBPs. The presence of HA tag sequences was confirmed by PCR. Subsequently, HA tag expression as part of CtrA and Tr1 proteins was confirmed by Western blot analysis using an Anti-HA tag antibody. We then used the Anti-HA tag antibody in ChIP assays to pull down *E. chaffeensis* DNA fragments interacting with the tagged DBPs, CtrA and Tr1 in two separate experiments. DNA segments pulled down with CtrA or Tr1 were identified by next-generation sequencing analysis (ChIP-seq analysis). These ChIP-seq results allowed us to show interactions with 189 and 101 genomic regions of *E. chaffeensis* significantly bound by CtrA and Tr1, respectively. The identified DNA-protein interactions were primarily present in intergenic regions of the *E. chaffeensis* genome where gene promoters exist. We then used CtrA and Tr1 recombinant proteins to confirm specific interactions with promoter regions selected from the ChIP-seq analysis. Specific interactions with 20 promoter regions for Tr1 and 19 promoter regions for CtrA were verified by EMSA experiments. This work-in-progress study demonstrates for the first time that CtrA and Tr1 are two global regulators, similar to EcxR in *E. chaffeensis*. Additional work is underway for this study and will continue during the next year funding period.

Specific Aim 2: Characterize the functional significance of genes identified as essential for *E. chaffeensis in vivo* growth.

We proposed two sub aims under this aim. During this funding period, we primarily focused in addressing the goals of *sub-aim 2.1 to characterize the genomic region spanning ECH_0659-ECH_0665 genes (the 7-gene segment)*, while also extending studies in the second sub-aim. Our prior studies identified three *E. chaffeensis* genes (ECH_0230, ECH_0379 and ECH_0660) as essential for the pathogen's persistent growth *in vivo*. We assessed Ech_0379 and Ech_0660 mutant organisms as vaccine candidates; infection with Ech_0379 mutant offered moderate protection, whereas the Ech_0660 mutant offered complete protection against wild type infection challenge. Differences in the host responses following vaccinations with Ech_0379 or Ech_0660 mutant were also documented after challenges with wild type infections in the canine host infection model (Nair *et al.* 2015 and McGill *et al.* 2016). We subsequently tested the impact of the Ech_0230 mutant, as it is one of the genes we identified earlier as essential for the pathogen's continued survival *in vivo* (Cheng *et al.* 2013). The Ech_0230 gene mutant cleared rapidly from the canine host (tested in five animals) and induced *E. chaffeensis*-specific antibody response. However, *E. chaffeensis*-specific $CD4_+$ T-cell proliferation and cytokine responses were not detected following vaccination with the Ech_0230 mutant. Further, this mutant did not confer protection against wild type *E. chaffeensis* challenge. These data demonstrate that host responses vary for different attenuated mutants of *E. chaffeensis.* This work was published recently (McGill et al. 2018).

We then pursued the investigations regarding why the attenuated mutant having an insertion mutation in ECH_0660 offers the best protection and its importance to *E. chaffeensis* persistent growth *in vivo*. We evaluated the impact of mutations in ECH_0379 and ECH_0660 on the proteome compared to wild type *E. chaffeensis.* Mutation within an antiporter gene (ECH_0379) resulted in drastic down regulation of many expressed proteins leading to metabolic depression. Mutation within a predicted phage structural protein gene (ECH_0660), however, resulted in fewer changes in the pathogen proteome, thus keeping its integrity very similar to the wild-type *E. chaffeensis* (Kondethimmanahalli *et al.* 2019). Together, these data suggest that the ECH_0660 mutant, while losing its ability to persist in a host, offers the best protection against infection challenge because of its extensive proteome conservation and thus simulating the phenotype similar to wild-type.

Bioinformatic analysis of the genomic region surrounding ECH_0660 gene identified the presence of a cluster of 7 genes spanning from ECH_0659 to ECH_0665. The 7-gene cluster includes four genes with homology to genes encoding for bacterial phage proteins. We also identified putative heme- and zinc-binding sites within the ECH_0659 and ECH_0661 gene promoter regions, respectively. We created transcriptional fusions in *E. coli* containing various segments of the upstream promoter region starting from ECH_0659 gene start codon. The constructs responded to zinc and iron starvation by enhancing the transcript levels compared to controls. Consistent with these data, transcripts for the seven genes in *E. chaffeensis* were also significantly higher in the ECH_0660 mutant compared to wild type. In contrast, the

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RNA expression levels for all seven genes in our newly developed ECH_0665 mutant showed significantly lower expression compared to the wild-type *E. chaffeensis*. These data suggest that ECH_0660 gene disruption affects *E. chaffeensis* metal ion homeostasis, thus making it inefficient at defending against the host innate immune response. The data from this portion of the work is compiled for publication. It will be submitted to a Unpublished ; manuscript in progress).

Specific Aim 3: Mutagenesis and *in vivo* screening in defining genomic regions critical for the bacterial pathogenesis in vertebrate and tick hosts.

In this aim, we proposed to generate random mutagenesis library to identify many *E. chaffeensis* genes and genomic regions critical for the pathogen by performing *in vivo* infection experiments. We reported earlier that Himar1 transposon-based random mutagenesis is a valuable tool in defining *E. chaffeensis* genes critical for its persistent growth *in vivo* in reservoir and incidental hosts (Cheng *et al.* 2013). The method also aided in extending studies focused on vaccine development and immunity (Nair *et al.* 2015 and McGill *et al.* 2016). To extend these investigations, we have generated a mutagenesis library having a large pool of mutants. As part of the current investigation, we mapped 55 new mutations and their genomic locations were established by Southern blot analysis, insertion region-specific PCR and sequencing of the amplicons. To define the critical nature of the bacterial genes, infection experiments were carried out in the canine host with pools of mutant organisms. Infection evaluation in this physiologically relevant host was performed by molecular assays to monitor the circulating mutants in blood and by xenodiagnosis to determine which persistent mutants are acquired by *Amblyomma americanum* ticks. The detailed investigation allowed the identification of many proteins critical for the pathogen's persistent *in vivo* growth. Enzymes/proteins involved in biotin biosynthesis, protein and fatty acid biosynthesis, DNA repair, electron transfer, and a component of an MDR efflux pump are among the proteins identified as essential for the pathogen's *in vivo* growth. Three known immunodominant membrane proteins, i.e., two p28-Omps and a 120 kDa surface protein, are also recognized as necessary for the pathogen's obligate intracellular lifecycle. The discovery of many *E. chaffeensis* proteins crucial for its continuous *in vivo* growth will serve as a major resource for future investigations aimed at defining pathogenesis and developing novel therapeutics for this and related *Anaplasmataceae* pathogens. These data are compiled for publication and submitted to a Unpublished Unpublished manuscript in review).

C. SIGNIFICANCE: We continue to investigate addressing our central hypothesis; *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals and that host cell-specific gene expression is essential for the pathogen's continued survival in vertebrate and tick cell environments. We established innovative strategies in defining the molecular basis of differential gene expression by mapping promoters of several genes of *E. chaffeensis* genes. We also extended studies in determining the importance of proteins critical for the pathogen's successful invasion and persistence in vertebrate hosts. We took advantage of our established mutagenesis methods to continue defining the critical genomic regions of the pathogen. Our studies continue to pave the way for both basic and applied research goals in defining the pathogenesis, host immunity required to protect against infections, and the development of novel therapeutics and vaccines. Our studies, therefore, continue to be highly innovative and impactful in offering new directions to study rickettsial diseases impacting the health of people and various vertebrate hosts.

D. PLANS: We will continue our work on all three specific aims of the proposal to further the progress on the proposed goals. We will continue our studies in evaluating the five identified DBPs, and also initiate research on sub-aims 1.1 and 1.2 with a focus to evaluate additional *E. chaffeensis* DBPs expressed when the pathogen is replicating in tick cell and macrophage environments during its developmental stages; reticulate cells and dense core cells. Efforts will also be under way to map variations in the DBPs assessed by RNA and protein analysis during the course of the pathogen developmental stages in tick and macrophage cell environments. We will also extend studies under specific aim 2 in defining the functional significance of genes identified as essential for *E. chaffeensis in vivo* growth, including ECH_0379 and ECH_0660.

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E. PUBLICATIONS:

1) Sequence Determinants Spanning -10 Motif and Spacer Region Implicated in Unique Ehrlichia chaffeensis Sigma 32-Dependent Promoter Activity of dnaK Gene. Liu H, Ganta RR. *Front Microbiol.* 2019 Aug 2;10:1772. doi: 10.3389/fmicb.2019.01772. eCollection 2019.

2) Proteome analysis revealed changes in protein expression patterns caused by mutations in *Ehrlichia chaffeensis*. Kondethimmanahalli C, Liu H, Ganta RR. *Front Cell Infect Microbiol.* 2019 Mar 18;9:58. doi: 10.3389/fcimb.2019.00058. eColle DnaK ction 2019.

3) Antigen-Specific CD4+CD8+ Double-Positive T Cells Are Increased in the Blood and Spleen During Ehrlichia chaffeensis Infection in the Canine Host. McGill JL, Wang Y, Ganta CK, Boorgula GDY, Ganta RR. *Front Immunol.* 2018 Jul 11;9:1585. doi: 10.3389/fimmu.2018.01585. eCollection 2018.

4) Current and Future Distribution of the Lone Star Tick, *Amblyomma americanum* (L.) (Acari: Ixodidae) in North America. Raghavan RK, Peterson T, Cobos M, Ganta RR, and Foley D. *PLoS One* 14(1): e0209082, 2019.

F. ABSTRACTS:

1) Sequence determinants spanning -10 motif and spacer region implicated in unique *Ehrlichia chaffeensis* sigma 32-dependent promoter activity of DnaK gene. Huitao Liu and Roman R. Ganta. International Conference on Biotechnological Interventions for Societal Development (BioSangam 2020), Prayagraj, India; February 21-23, 2020.

2) Sequence determinants spanning -10 motif and spacer region implicated in unique *Ehrlichia chaffeensis* sigma 32-dependent promoter activity of Dnak gene. Huitao Liu and Roman R. Ganta. Current Trends in Pharmaceutical and Medical Sciences conference held at Kashipur, India; February 26-28, 2020.

3) Mutagenesis aids in studying pathogenesis and vaccine development against *Ehrlichia* and *Anaplasma* species pathogens impacting human and animal health. Roman R. Ganta. Recent Advances in Chemical, Pharmaceutical and Biological Sciences conference, Guntur, India; March 5-7, 2020.

G. PATENTS:

None.

Obtained via FOIA by White Coat Waste Project

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Ganta RR, Cheng C, Miller EC, McGuire BL, Peddireddi L, Sirigireddy KR, Chapes SK. Differential clearance and immune responses to tick cell-derived versus macrophage culture-derived Ehrlichia chaffeensis in mice. Infection and immunity. 2007 January;75(1):135-45. PubMed PMID: 17060466; PubMed Central PMCID: PMC1828415; DOI: 10.1128/IAI.01127-06.
Complete	Luce-Fedrow A, Von Ohlen T, Boyle D, Ganta RR, Chapes SK. Use of Drosophila S2 cells as a model for studying Ehrlichia chaffeensis infections. Applied and environmental microbiology. 2008 March;74(6):1886-91. PubMed PMID: 18245255; PubMed Central PMCID: PMC2268305; DOI: 10.1128/AEM.02467-07.
Non-Compliant	Cheng C, Ganta RR. Laboratory maintenance of Ehrlichia chaffeensis and Ehrlichia canis and recovery of organisms for molecular biology and proteomics studies. Current protocols in microbiology. 2008 May;Chapter 3:Unit 3A.1. PubMed PMID: 18770537; DOI: 10.1002/9780471729259.mc03a01s9.
Non-Compliant	Cheng C, Sirigireddy KR, Ganta RR. Isolation and molecular detection of Ehrlichia from vertebrate animals. Current protocols in microbiology. 2008 May;Chapter 3:Unit 3A.3. PubMed PMID: 18770538; DOI: 10.1002/9780471729259.mc03a03s9.
Complete	Seo GM, Cheng C, Tomich J, Ganta RR. Total, membrane, and immunogenic proteomes of macrophage- and tick cell-derived Ehrlichia chaffeensis evaluated by liquid chromatography-tandem mass spectrometry and MALDI-TOF methods. Infection and immunity. 2008 November;76(11):4823-32. PubMed PMID: 18710870; PubMed Central PMCID: PMC2573352; DOI: 10.1128/IAI.00484-08.
Complete	Ganta RR, Peddireddi L, Seo GM, Dedonder SE, Cheng C, Chapes SK. Molecular characterization of Ehrlichia interactions with tick cells and macrophages. Frontiers in bioscience (Landmark edition). 2009 January 1;14:3259-73. PubMed PMID: 19273271; PubMed Central PMCID: PMC4392924; DOI: 10.2741/3449.
Complete	Peddireddi L, Cheng C, Ganta RR. Promoter analysis of macrophage- and tick cell-specific differentially expressed Ehrlichia chaffeensis p28-Omp genes. BMC microbiology. 2009 May 19;9:99. PubMed PMID: 19454021; PubMed Central PMCID: PMC2694197; DOI: 10.1186/1471-2180-9-99.
Complete	Reinbold JB, Coetzee JF, Sirigireddy KR, Ganta RR. Detection of Anaplasma marginale and A. phagocytophilum in bovine peripheral blood samples by duplex real-time reverse transcriptase PCR assay. Journal of clinical microbiology. 2010 July;48(7):2424-32. PubMed PMID: 20463162; PubMed Central PMCID: PMC2897487; DOI: 10.1128/JCM.02405-09.
Non-Compliant	Reinbold JB, Coetzee JF, Hollis LC, Nickell JS, Riegel C, Olson KC, Ganta RR. The efficacy of three chlortetracycline regimens in the treatment of persistent Anaplasma marginale infection. Veterinary microbiology. 2010 September 28;145(1-2):69-75. PubMed PMID: 20346598; DOI: 10.1016/j.vetmic.2010.02.031.
Non-Compliant	Reinbold JB, Coetzee JF, Hollis LC, Nickell JS, Riegel CM, Christopher JA, Ganta RR. Comparison of iatrogenic transmission of Anaplasma marginale in Holstein steers via needle and needle-free injection techniques. American journal of veterinary research. 2010 October;71(10):1178-88. PubMed PMID: 20919904; DOI: 10.2460/ajvr.71.10.1178.
Complete	Von Ohlen T, Luce-Fedrow A, Ortega MT, Ganta RR, Chapes SK. Identification of critical host mitochondrion-associated genes during Ehrlichia chaffeensis infections. Infection and immunity. 2012 October;80(10):3576-86. PubMed PMID: 22851751; PubMed Central PMCID: PMC3457586; DOI: 10.1128/IAI.00670-12.

Obtained via FOIA by White Coat Waste Project

Complete	Stoffel RT, McClure JC, Butcher MM, Johnson GC, Roland W, Cheng C, Sirigireddy KR, Ganta R, Boughan K, Ewing SA, Stich RW. Experimental infection of Rhipicephalus sanguineus with Ehrlichia chaffeensis. Veterinary microbiology. 2014 August 6;172(1-2):334-8. PubMed PMID: 24894131; PubMed Central PMCID: PMC4098862; DOI: 10.1016/j.vetmic.2014.04.017.
Non-Compliant	Wilkerson MJ, Black KE, Lanza-Perea M, Sharma B, Gibson K, Stone DM, George A, Nair AD, Ganta RR. Initial development and preliminary evaluation of a multiplex bead assay to detect antibodies to Ehrlichia canis, Anaplasma platys, and Ehrlichia chaffeensis outer membrane peptides in naturally infected dogs from Grenada, West Indies. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 2017 January;29(1):109-114. PubMed PMID: 27852813; DOI: 10.1177/1040638716671979.
Complete	McClure EE, Chávez ASO, Shaw DK, Carlyon JA, Ganta RR, Noh SM, Wood DO, Bavoil PM, Brayton KA, Martinez JJ, McBride JW, Valdivia RH, Munderloh UG, Pedra JHF. Engineering of obligate intracellular bacteria: progress, challenges and paradigms. Nature reviews. Microbiology. 2017 September;15(9):544-558. PubMed PMID: 28626230; PubMed Central PMCID: PMC5557331; DOI: 10.1038/nrmicro.2017.59.
Complete	Wang Y, Wei L, Liu H, Cheng C, Ganta RR. A genetic system for targeted mutations to disrupt and restore genes in the obligate bacterium, Ehrlichia chaffeensis. Scientific reports. 2017 November 17;7(1):15801. PubMed PMID: 29150636; PubMed Central PMCID: PMC5693922; DOI: 10.1038/s41598-017-16023-y.
Non-Compliant	Kim D, Jaworski DC, Cheng C, Nair AD, Ganta RR, Herndon N, Brown S, Park Y. The transcriptome of the lone star tick, Amblyomma americanum, reveals molecular changes in response to infection with the pathogen, Ehrlichia chaffeensis. Journal of Asia-Pacific Entomology. 2018 September;21(3):852-863. DOI: 10.1016/j.aspen.2018.05.009.
Complete	Kondethimmanahalli C, Ganta R. Impact of Three Different Mutations in Ehrlichia chaffeensis in Altering the Global Gene Expression Patterns. Scientific reports. 2018 April 18;8(1):6162. PubMed PMID: 29670161; PubMed Central PMCID: PMC5906474; DOI: 10.1038/s41598-018-24471-3.
Complete	Eedunuri VK, Zhang Y, Cheng C, Chen L, Liu H, Omsland A, Boyle D, Ganta RR. Protein and DNA synthesis demonstrated in cell-free Ehrlichia chaffeensis organisms in axenic medium. Scientific reports. 2018 June 18;8(1):9293. PubMed PMID: 29915240; PubMed Central PMCID: PMC6006305; DOI: 10.1038/s41598-018-27574-z.
Complete	McGill JL, Wang Y, Ganta CK, Boorgula GDY, Ganta RR. Antigen-Specific CD4⁺CD8⁺ Double-Positive T Cells Are Increased in the Blood and Spleen During Ehrlichia chaffeensis Infection in the Canine Host. Frontiers in immunology. 2018 July 11;9:1585. PubMed PMID: 30050533; PubMed Central PMCID: PMC6050357; DOI: 10.3389/fimmu.2018.01585.
Complete	Kondethimmanahalli C, Liu H, Ganta RR. Proteome Analysis Revealed Changes in Protein Expression Patterns Caused by Mutations in Ehrlichia chaffeensis. Frontiers in cellular and infection microbiology. 2019;9:58. PubMed PMID: 30937288; PubMed Central PMCID: PMC6431617; DOI: 10.3389/fcimb.2019.00058.
Complete	Liu H, Ganta RR. Sequence Determinants Spanning -10 Motif and Spacer Region Implicated in Unique Ehrlichia chaffeensis Sigma 32-Dependent Promoter Activity of dnaK Gene. Frontiers in microbiology. 2019;10:1772. PubMed PMID: 31428069; PubMed Central PMCID: PMC6687850; DOI: 10.3389/fmicb.2019.01772.
Complete	Raghavan RK, Peterson AT, Cobos ME, Ganta R, Foley D. Current and Future Distribution of the Lone Star Tick, Amblyomma americanum (L.) (Acari: Ixodidae) in North America. PloS one. 2019;14(1):e0209082. PubMed PMID: 30601855; PubMed Central PMCID: PMC6314611; DOI: 10.1371/journal.pone.0209082.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

Obtained via FOIA by White Coat Waste Project

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

Obtained via FOIA by White Coat Waste Project

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	GANTA, ROMAN R.	BS,MS,PHD	PD/PI	EFFORT					NA
	N	Redacted by agreement	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N		PHD	Staff scientist (Doctoral level)						NA
	N		PHD	Staff scientist (Doctoral level)						NA
	N		PHD	Co-Investigator						NA
	N		PHD,BS,MS	Co-Investigator						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

Obtained via FOIA by White Coat Waste Project

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

Obtained via FOIA by White Coat Waste Project

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

We continue to investigate addressing our central hypothesis; E. chaffeensis differentially regulates its gene expression in response to host cell environmental signals and that host cell-specific gene expression is essential for the pathogen's continued survival in vertebrate and tick cell environments. We established innovative strategies in defining the molecular basis of differential gene expression by mapping promoters of several genes of E. chaffeensis genes. We also extended studies in determining the importance of proteins critical for the pathogen's successful invasion and persistence in vertebrate hosts. We took advantage of our established mutagenesis methods to continue defining the critical genomic regions of the pathogen. Our studies continue to pave the way for both basic and applied research goals in defining the pathogenesis, host immunity required to protect against infections, and the development of novel therapeutics and vaccines. Our studies, therefore, continue to be highly innovative and impactful in offering new directions to study rickettsial diseases impacting the health of people and various vertebrate hosts.

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

Obtained via FOIA by White Coat Waste Project

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

Obtained via FOIA by White Coat Waste Project

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Kansas State University	929773554	KS-001	Kansas State University 2 Fairchild Hall Manhattan KS 665061103
KANSAS STATE UNIVERSITY	929773554		KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL, 1601 VATTIER STREET MANHATTAN KS 665062504
Kansas State University	929773554	KS-001	Kansas State University 2 Fairchild Hall Manhattan KS 66506

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

Obtained via FOIA by White Coat Waste Project

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Obtained via FOIA by White Coat Waste Project

Recipient Information

1. Recipient Name
KANSAS STATE UNIVERSITY
2 FAIRCHILD HALL

MANHATTAN, KS 66506

2. Congressional District of Recipient
01

3. Payment System Identifier (ID)
1480771751A1

4. Employer Identification Number (EIN)
480771751

5. Data Universal Numbering System (DUNS)
929773554

6. Recipient's Unique Entity Identifier

7. Project Director or Principal Investigator
ROMAN R. GANTA, PHD
Professor
rganta@vet.k-state.edu
785-532-4612

8. Authorized Official
Paul R. Lowe
plowe@ksu.edu
(785) 532-6804

Federal Agency Information

9. Awarding Agency Contact Information
Parvathy Rajeev Panakal

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
parvathy.panakal@nih.gov
240-627-3505

10. Program Official Contact Information
Samuel S. Perdue
Program Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
sperdue@niaid.nih.gov
240-627-3341

Federal Award Information

11. Award Number
5R01AI070908-12

12. Unique Federal Award Identification Number (FAIN)
R01AI070908

13. Statutory Authority
42 USC 241 42 CFR 52

14. Federal Award Project Title
Vector and Host Contributions to the Regulation of E. Chaffeensis Gene Expression

15. Assistance Listing Number
93.855

16. Assistance Listing Program Title
Allergy and Infectious Diseases Research

17. Award Action Type
Non-Competing Continuation

18. Is the Award R&D?
Yes

Summary Federal Award Financial Information

19. Budget Period Start Date 07/01/2021 **– End Date** 06/30/2022	
20. Total Amount of Federal Funds Obligated by this Action	$536,311
20 a. Direct Cost Amount	$352,836
20 b. Indirect Cost Amount	$183,475
21. Authorized Carryover	$0
22. Offset	$0
23. Total Amount of Federal Funds Obligated this budget period	$536,311
24. Total Approved Cost Sharing or Matching, where applicable	$0
25. Total Federal and Non-Federal Approved this Budget Period	$536,311
26. Project Period Start Date 12/15/2007 **– End Date** 06/30/2024	
27. Total Amount of the Federal Award including Approved Cost Sharing or Matching this Project Period	$1,608,933

28. Authorized Treatment of Program Income
Additional Costs

29. Grants Management Officer - Signature
Regina E. Kitsoulis

30. Remarks

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

Obtained via FOIA by White Coat Waste Project

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RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

SECTION I – AWARD DATA – 5R01AI070908-12

Principal Investigator(s):
ROMAN R. GANTA, PHD

Award e-mailed to: ksuawards@ksu.edu

Dear Authorized Official:

The National Institutes of Health hereby awards a grant in the amount of $536,311 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to KANSAS STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI070908. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please direct questions to the Federal Agency contacts.

Sincerely yours,

Regina E. Kitsoulis
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Cumulative Award Calculations for this Budget Period (U.S. Dollars)

Obtained via FOIA by White Coat Waste Project
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Federal Direct Costs	$352,836
Federal F&A Costs	$183,475
Approved Budget	$536,311
Total Amount of Federal Funds Authorized (Federal Share)	$536,311
TOTAL FEDERAL AWARD AMOUNT	$536,311
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$536,311

SUMMARY TOTALS FOR ALL YEARS (for this Document Number)		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
12	$536,311	$536,311
13	$536,311	$536,311
14	$536,311	$536,311

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

Payment System Identifier:	1480771751A1
Document Number:	RAI070908D
PMS Account Type:	P (Subaccount)
Fiscal Year:	2021

IC	CAN	2021	2022	2023
AI	8472364	$536,311	$536,311	$536,311

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M46C B / **OC**: 41025 / **Released**: Kitsoulis, Regina 07/07/2021
Award Processed: 07/08/2021 12:16:51 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI070908-12

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – STANDARD TERMS AND CONDITIONS – 5R01AI070908-12

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH

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awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI070908. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:

Additional Costs

SECTION IV – AI SPECIFIC AWARD CONDITIONS – 5R01AI070908-12

Clinical Trial Indicator: No

This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

Select Agents:

Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or

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biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

SPREADSHEET SUMMARY
AWARD NUMBER: 5R01AI070908-12

INSTITUTION: KANSAS STATE UNIVERSITY

Facilities and Administrative Costs	Year 12	Year 13	Year 14
F&A Cost Rate 1	52%	52%	52%
F&A Cost Base 1	$352,836	$352,836	$352,836
F&A Costs 1	$183,475	$183,475	$183,475

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A. COVER PAGE

Project Title: Vector and Host Contributions to the Regulation of E. Chaffeensis Gene Expression	
Grant Number: 5R01AI070908-12	**Project/Grant Period:** 12/15/2007 - 06/30/2024
Reporting Period: 07/01/2020 - 06/30/2021	**Requested Budget Period:** 07/01/2021 - 06/30/2022
Report Term Frequency: Annual	**Date Submitted:** 05/13/2021
Program Director/Principal Investigator Information: ROMAN R GANTA , PHD MS BS **Phone Number:** 785-532-4612 **Email:** rganta@vet.k-state.edu	**Recipient Organization:** KANSAS STATE UNIVERSITY KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL, 1601 VATTIER STREET MANHATTAN, KS 665062504 **DUNS:** 929773554 **EIN:** 1480771751A1 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu	**Signing Official:** PAUL R LOWE 2 Fairchild Hall Manhattan, KS 665101103 **Phone number:** 785-532-6804 **Email:** plowe@ksu.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** Yes **If yes, previously reported:** Yes

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B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS: Rickettsial diseases caused by the Anaplasmataceae family pathogens of the genera Ehrlichia and Anaplasma remain a growing public health concern spanning the past three decades, and are also the second leading cause of tick-borne infections in humans throughout the USA and many parts of the world. The diseases include human monocytic and granulocytic ehrlichiosis caused by Ehrlichia chaffeensis and Ehrlichia ewingii respectively, and human granulocytic anaplasmosis resulting from Anaplasma phagocytophilum infections. Recently, another tick-borne pathogen, Ehrlichia muris subsp. eauclairensis, has been identified as causing another ehrlichial disease in people. Despite the complex cellular environments of arthropods and vertebrates having sophisticated systems of defense, these pathogens have evolved strategies to evade host clearance. For example, E. chaffeensis in macrophages and tick cells has distinct patterns of protein expression and the pathogen translocates effectors in altering host responses to support its continued survival in a host. Our prior studies demonstrated that differential expression in vertebrate and tick cells alters host responses leading to variations in vertebrate host clearance/persistence. The central hypothesis of our competing renewal application remains that E. chaffeensis differentially regulates its gene expression in response to host cell environmental signals and that the host cell-specific gene expression is essential for the pathogen's continued survival in vertebrate and tick cell environments. Specific aims of the previous funded project are to 1) characterize the E. chaffeensis RNA polymerase complex in understanding the pathogen's host-specific differential gene expression; 2) evaluate the significance of host-specific differential expression by characterizing mutations in three genes identified as essential for the in vivo growth of E. chaffeensis; and 3) perform mutational analysis and in vivo screening to identify additional genes essential for E. chaffeensis pathogenesis in vertebrate and tick hosts. We made substantial progress in addressing all three specific aims. The progress, as evidenced in numerous peer-reviewed publications (24 peer-reviewed publications and 7 reviews/book chapters), paved the way for new directions of this renewal application. The new specific aims of this renewal application continue to address the above stated hypothesis. The proposed aims are supported with a strong premise. Furthermore, the proposed project goals are highly innovative and attempt to fill-in a significant gap of knowledge. We are the only research group investigating the detailed regulation of gene expression in Ehrlichia species and such studies may be extended in other Ehrlichia and Anaplasma species pathogens. We also lead the investigations on targeted mutagenesis studies for Anaplasmataceae pathogens, including in creating mutations disrupting and restoring a gene function. We used the methods in studies to understand pathogenesis and in studying gene regulation in Ehrlichia species. Targeted mutations to disrupt and also to functionally restore a gene will be valuable in testing molecular Koch's postulates. We have also created targeted mutations to generate bacterial fusion proteins, which are important in studies to characterize E. chaffeensis proteins in vivo. Additionally, we developed physiologically relevant animal infection models. Moreover, we standardized tick transmission methods in support of understanding the bacterial pathogenesis, host responses and vaccine development. We also extended studies in developing an attenuated vaccine to protect an incidental host from E. chaffeensis by needle and tick transmitted challenge. The in vivo infection models will also be valuable in investigating the importance of genes to the E. chaffeensis life cycle in tick and vertebrate hosts. Collectively, the extensive novel data, generated with the support of our previous R01 grant, form a strong foundation for the proposed three specific aims of this renewal application.

Specific Aims:
1) Characterize sequence-specific DNA binding proteins (DBPs) in E. chaffeensis impacting RNA polymerase function in support of understanding the pathogen's host-specific differential gene expression.
2) Characterize the functional significance of genes identified as essential for E. chaffeensis in vivo growth.
3) Mutagenesis and in vivo screening in defining genomic regions critical for the bacterial pathogenesis in vertebrate and tick hosts.

The proposed specific aims have a strong scientific premise and are a logical extension of the substantial progress we made during the previous funding cycle. The project goals are supported by the research data reported in our several peer-reviewed publications and with the inclusion of additional data. We have sufficient expertise to ensure successful execution of the project, and we included well thought-out authentication plans. All molecular tools to study gene expression were developed by us. We also have accomplished mutagenesis and in vivo screening methods to test the importance of E. chaffeensis genes in tick and vertebrate hosts. We believe that the goals of this renewal project are also critical in advancing understanding of

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the Ehrlichia pathogenesis. We expect that our continued progress will pave the way for similar studies in related rickettsials in controlling diseases caused by Anaplasma, Ehrlichia and Neorickettsia species.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File Uploaded : Ganta NIH R01 Progress report May 13 2021.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Aim 1) The work under sub-aims 1.1, 1.2 and 1.3 will continue. Under sub-aim 1.1, we will perform electromobility shift assays (EMSA) and DNAse I foot printing experiments, coupled with mass-spectrometry analysis to identify DNA binding proteins (DBPs) interacting with the three gene promoters of p28-Omp 14 and 19 genes and DnaK gene. As part of sub-aim 1.2, we designed TaqMan primers and probes for defining transcriptional variations for the five predicted DBP genes (EcxR, HU, CtrA, MerR and Tr1) for use in the qRT-PCR analysis. Sub-aim 1.3 research will extend in performing Chromatin immunoprecipitation (ChiP) experiments using the HA-tagged mutant E. chaffeensis for EcxR, MerR, and HU. The protocols for these experiments will be followed as per the ChiP assays already performed for CtrA and Tr1 interactions with the genomic regions.

Aim 2) The ongoing work in defining the functional significance of the phage proteins and antiporter proteins for E. chaffeensis will continue. Specifically, we plan to extend investigations in identifying interacting proteins of the pathogen with the phage head-to-tail connector protein (ECH_0660 gene product) and similarly the sodium/proton antiporter protein (ECH_0379 gene product). The studies will utilize both wild type and the mutant organisms with functional disruption mutations in ECH_0379 and ECH_0660.

Aim 3) The discovery of many E. chaffeensis proteins crucial for its continuous in vivo growth which we reported recently serves as a major resource for investigations aimed at understanding the bacterial biology and in defining how they may contribute to pathogenesis. Research will now be extended in addressing these goals.

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A. SPECIFIC AIMS: This project is focused to investigate how *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals for its survival in vertebrate and tick cell environments. The project has the following three specific aims:

1) *Characterize sequence-specific DNA binding proteins (DBPs) in E. chaffeensis impacting RNA polymerase function in support of understanding the pathogen's host-specific differential gene expression.*

2) *Characterize the functional significance of genes identified as essential for E. chaffeensis in vivo growth.*

3) *Mutagenesis and in vivo screening to define genomic regions critical for the bacterial pathogenesis in vertebrate and tick hosts.*

B. STUDIES AND RESULTS:

Specific Aim 1: ***Characterize sequence-specific DNA binding proteins (DBPs) in E. chaffeensis impacting RNA polymerase function in support of understanding the pathogen's host-specific differential gene expression.*** This aim has three sub-aims. We initiated the work on all three sub-aims and have made substantial progress.

Sub-aim 1.1) Evaluation of *E. chaffeensis* lysates prepared from tick cell (ISE6) and macrophage (DH82) culture-derived RC and DC fractions. We improved the protocols for recovering large quantities of *E. chaffeensis* DC and RC fractions by density gradient centrifugation methods and isolated large quantities of the two different forms of *E. chaffeensis* from the ISE6 and DH82 cultures. The purified proteins are now being investigated to identify differential expression of DBPs in the four different fractions of the organism. Further, we generated polyclonal sera raised against all five DBPs in rabbits and the antibodies are purified using protein A sepharose IgG purification columns. The antibodies will be used in experiments to define the protein expression variations by Western blot analysis and for co-immunoprecipitation experiments. This work is being pursued by a graduate student and the student research training is part of this sub-aim. The student will perform electromobility shift assays (EMSA) and DNAse I foot printing experiments, coupled with mass-spectrometry analysis to identify DBPs interacting with the three gene promoters of p28-Omp 14 and 19 genes and DnaK gene. This work will continue as part of the next year's research goals.

Sub-aim 1.2) RNA and protein analysis to map variations in DBPs expressions. In support of this sub-aim, we similarly cultured large quantities of *E. chaffeensis* in ISE6 and DH82 cultures and the bacterial RNAs were recovered from different time points during the cell cultures, as well as from the DC and RC fractions. This work is being undertaken by another graduate student, and she is currently processing the RNA for assessing the transcriptional variations by real-time quantitative RT-PCR (qRT-PCR) analysis for the transcripts for DBPs; EcxR, HU, CtrA, MerR and Tr1. TaqMan primers and probes were designed for the gene transcripts of the five DBPs for use in the qRT-PCR analysis. This work is currently ongoing and will continue as part of the next year's project goals.

Sub-aim 1.3) Targeted mutagenesis to generate fusion tags co-expressed with the five predicted DBPs for monitoring DNA-protein interactions. *E. chaffeensis* gene regulation in the tick and vertebrate hosts is currently poorly understood. *E. chaffeensis* DBPs; EcxR, Tr1, CtrA, MerR and HU were selected for the tagged mutagenesis experiments. EcxR has a DNA-binding winged helix-turn-helix (HTH) motif. Tr1 homologs are known to be involved in the xenobiotic response with the cro/C1-type HTH DNA-binding domain. CtrA homologs are known to be involved in two-component signal transduction systems with a DNA binding domain located at the C-terminal end. MerR, as part of the MerR family transcription regulators, mediates responses to stress including exposure to heavy metals, drugs, or oxygen free radicals and regulates transcription of multidrug/metal ion transporter genes and oxidative stress regulons. HU is predicted to be a histone-like protein, which plays a critical role in global regulation of transcription by binding at various locations on genomic DNA in bacteria. We created tagged mutations with hemagglutinin (HA) tags expressed as part of the proteins at the c-terminal ends. The goal is to use the HA tag-specific antibodies in chromatin immune precipitation (ChiP) experiments to allow identifying genes interacting with the DBPs. We performed ChiP analysis to pull down *E. chaffeensis* DNA fragments interacting with CtrA and Tr1. DNA segments pulled down with CtrA or Tr1 were identified by sequencing analysis. The results

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allowed us to define the interactions at nearly 300 genomic regions of *E. chaffeensis* bound by CtrA and Tr1. Specific interactions with 39 promoter regions (20 for Tr1 and 19 for CtrA) were verified by EMSA experiments (Unpublished 2021, manuscript in progress). We also assessed all five DBPs for their roles in influencing the promoter activities of 7 selected genes coding for *ClpB, DnaK, GroES/L, HU, p28-Omp 14, p28-Omp 19,* and *Tr1*. Specific interactions of all 7 promoters with EcxR were observed independently by EMSA, South-Western blot analysis and using a Yeast hybrid system. This work is also being compiled for publication (Unpublished manuscript in progress). Together, these studies allowed the first description of CtrA and Tr1 as global regulators, while also confirming the previous reports that EcxR is also a global regulator in *E. chaffeensis*. Similar ChiP experiments are in progress with EcxR, MerR, and HU and will be part of next year's project goals.

Specific Aim 2: ***Characterize the functional significance of genes identified as essential for E. chaffeensis in vivo growth. We proposed two sub aims under this aim.***

Sub-aim 2.1) Characterize the genomic region spanning ECH_0659-ECH_0665 genes (the 7-gene segment). Our prior studies identified three *E. chaffeensis* genes (ECH_0230, ECH_0379 and ECH_0660) as essential for the pathogen's persistent growth *in vivo*. We assessed all three gene mutants as potential live attenuated vaccine candidates; infection with the ECH_0230 offered no protection, the ECH_0379 mutant offered moderate protection, while the ECH_0660 mutant offered complete protection against wild type infection challenge (Nair *et al*. 2015 and McGill *et al.* 2016 and 2018). To define the molecular basis for these differences, we compared two mutants' transcriptomes and proteomes (ECH_0379 and ECH_0660) with those of wild type *E. chaffeensis.* Mutation within ECH_0379 encoding for an antiporter protein resulted in drastic downregulation of many expressed proteins leading to metabolic depression. Mutation within the ECH_0660 gene, which encodes for a phage head-to-tail connector protein, caused minimal changes, thus keeping its integrity very similar to the wild type *E. chaffeensis* (Kondethimmanahalli *et al*. 2018 and 2019). Together, these data suggest that the ECH_0660 mutant, while losing its ability to persist in a host, offers the best protection against infection challenge because of its extensive proteome conservation, which is similar to wild type *E. chaffeensis*. We then investigated why the function disruption mutation in ECH_0660 is detrimental for *E. chaffeensis* (Unpublished In press). We reported that the genomic region surrounding ECH_0660 gene included a cluster of 7 genes (ECH_0659 to ECH_0665) and the first four genes are part of an operon. The 7-gene cluster includes three additional genes having homology to genes encoding bacterial phage proteins. These genes are also conserved in several closely related rickettsia. Bacteriophages are known to be integrated in Gram-negative and Gram-positive bacterial genomes. Such integrated phage genes help pathogenic bacteria by serving as secretory systems for transport of nutrients and metabolites across bacterial membranes and in altering host response by translocating bacterial effectors. We, therefore, investigated if the 7-gene segment in *E. chaffeensis* contributes to nutritional transport. The promoter segment upstream to the first gene has putative heme- and zinc-binding sites. We created transcriptional fusions of the promoter segment in *E. coli*. The constructs responded to zinc and iron starvation by enhancing the transcript levels compared to controls. Consistent with these data, transcripts from the 7-gene cluster in *E. chaffeensis* cultured *in vitro* in macrophage cultures were also significantly higher in the ECH_0660 mutant compared to the wild type. In contrast, the RNA expression levels for all seven genes in our newly developed ECH_0665 mutant showed similar transcription levels or lower (for some genes) compared to the wild type *E. chaffeensis*. Deprivation of iron and zinc in the culture media caused enhanced transcription for both wild type and the mutants. Further, the starvation of the metal ions caused significant transcriptional upregulation from the 7-gene cluster for the ECH_0660 mutant. Together, these data suggest that the functional disruption in the predicted phage head-to-tail connector protein gene is likely detrimental by altering the pathogen's ability to obtain metal ions to support its growth within a phagosome of infected host macrophages. The function disruption mutation in the ECH_0660 gene, therefore, may have induced a metabolic burden for *E. chaffeensis* resulting in the upregulation of gene expression from several genes likely involved in the metal ion uptake. This work was accepted for (Unpublished).

Sub-aim 2.2) Evaluate sodium (cation)/proton antiporter genes in E. chaffeensis. Little is known about how *E. chaffeensis* regulates its intracellular pH when replicating within acidified phagosomes. Similarly, it is unclear how the DC form differs in pH homeostasis during the extracellular milieu. Sodium (cation): proton

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antiporter proteins exchange sodium or other monovalent cations across plasma membranes in regulating cytoplasmic pH levels. We reported earlier that a mutation in a Na^+/H^+ antiporter protein gene of *E. chaffeensis* (ECH_0379) caused reduced bacterial presence in vertebrate hosts. We then discovered that *E. chaffeensis* genome contains six genomic regions containing genes predicted to encode antiporter proteins. We developed a functional complementation assay in a sodium sensitive *E. coli* strain and used it to assess all predicted antiporter genes for their ability to complement the antiporter function. The *E. coli* system was used successfully in demonstrating that all predicted *E. chaffeensis* antiporter genes are functionally active and that they work differently in acidic and basic pH conditions. Further, we assessed the expression from all six genomic regions by RNA analysis performed by semi quantitative RT-PCR assays. Five of the six genomic regions containing antiporter protein genes were transcriptionally active, although expression is variable in tick cells and macrophage cultures. Further, we observed differences in DC and RC form of the bacterium. These data are now compiled for publication in a peer-reviewed journal Unpublished manuscript in progress).

Specific Aim 3: ***Mutagenesis and in vivo screening to define genomic regions critical for the bacterial pathogenesis in vertebrate and tick hosts.***

In this aim, we proposed to generate a random mutagenesis library to identify many *E. chaffeensis* genes and genomic regions critical for the pathogen's growth by performing *in vivo* infection experiments. We reported earlier that Himar1 transposon-based random mutagenesis is a valuable tool for defining *E. chaffeensis* genes critical for the pathogen's *in vivo* growth (Cheng *et al.* 2013). In support of the current aim, we have generated a mutagenesis library; we mapped 55 new mutations to define mutation sites within the *E. chaffeensis* genome by Southern blot analysis, and by insertion-specific PCRs and sequencing of the amplicons. To define the critical nature of the bacterial genes, *in vivo* infection experiments were also carried out in the canine host. The detailed investigation allowed the identification of many proteins critical for the pathogen's continued persistence in a host. Enzymes/proteins involved in biotin biosynthesis, protein and fatty acid biosynthesis, DNA repair, electron transfer, and a component of an MDR efflux pump are among the proteins identified as essential for the pathogen's *in vivo* growth. Three known immunodominant membrane proteins (two p28-OMPs and the120 kDa surface protein) are also recognized as necessary for the pathogen's obligate intracellular lifecycle. The discovery of many *E. chaffeensis* proteins crucial for its continuous *in vivo* growth will serve as a major resource for future investigations aimed at defining pathogenesis and developing novel therapeutics for this and related *Anaplasmataceae* pathogens. These data were published recently (Wang *et al.* 2020; *Infect. Immun.*).

Additional progress related to the project:

Zhang *et al.* Infect. Immun., 2021: The ability to grow obligate intracellular bacteria under axenic conditions can be a major advancement, as it will enable new paths of investigation, such as aiding the manipulation of the pathogenic organisms in the absence of host cells, clonal purification of bacterial mutants, and allowing detailed biochemical and genetic studies. As of now, such methods remain to be established for many obligate bacterial pathogens, including for several tick-borne rickettsia impacting human and animal health. We recently assessed the application of axenic media for *E. chaffeensis* where we successfully demonstrated protein and DNA biosynthesis for the RC form of the pathogen (Eedunuri et al. 2018). Considering the potential advancements likely achieved using the axenic media growth for the pathogenic rickettsials, we extended investigations to improve the axenic growth conditions for *E. chaffeensis* and also initiated similar experiments for *A. phagocytophilum*. The results were published recently in the journal, *Infect. Immun.* (Zhang *et al.* 2021), where we presented novel data demonstrating the purification of host cell-free phagosomes containing *A. phagocytophilum* or *E. chaffeensis* and used the phagosomes to assess the bacterial protein and DNA synthesis in axenic media conditions. In this study, we demonstrated protein and DNA biosynthesis *in vitro* in axenic media culture conditions in purified phagosome-containing bacteria. The study forms the strong foundation for furthering the possibility of fully developed methods in promoting sustained and enhanced replication of the pathogenic rickettsials in the absence of a host cell support. The host cell-free axenic growth of obligate *Anaplasmataceae* pathogens will be critical in advancing research goals in many important tick-borne diseases impacting human and animal health, including in supporting the project goals for the current project.

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C. SIGNIFICANCE: We continue to investigate our central hypothesis; *E. chaffeensis* differentially regulates its gene expression in response to host cell environmental signals and that host cell-specific gene expression is essential for the pathogen's continued survival in vertebrate and tick cell environments. We established innovative strategies in defining the molecular basis of differential gene expression by mapping promoters of several genes of *E. chaffeensis*. We also extended studies in determining the importance of proteins critical for the pathogen's successful invasion and persistence in vertebrate hosts. Our recently established mutagenesis methods facilitated greatly in defining the critical genomic regions of the pathogen. Our studies will continue to pave the way for impactful research goals in defining the pathogenesis, host immunity required to protect against infections, and in developing novel therapeutics and vaccines. Our studies, therefore, continue to be highly innovative and impactful in offering new directions to study rickettsial diseases impacting the health of people, companion animals, and agricultural animals.

D. PUBLICATIONS:

1. Mutations in *Ehrlichia chaffeensis* genes ECH_0660 and ECH_0665 cause transcriptional changes in response to Zinc or Iron limitation. Torres-Escobar A, Juárez-Rodríguez MD, and **Ganta RR.** ***J. Bacterial.,*** 2021 (https://jb.asm.org/content/early/2021/04/16/JB.00027-21).
2. Multiple *Ehrlichia chaffeensis* genes critical for its persistent infection in a vertebrate host are identified by random mutagenesis coupled with *in vivo* infection assessment. Wang Y, Nair ADS, Alhassan A, Jaworski DC, Liu H, Trinkl K, Hove P, Ganta CK, Burkhardt N, Munderloh UG and **Ganta RR**. ***Infect. Immun.*** 2020, 88:e00316-20. https://doi.org/10.1128/IAI.00316-20).
3. Molecular detection and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs from the Caribbean. Alhassan A, Hove P, Sharma B, Matthew-Belmar V, Karasek I, Lanza-Perea M, Werners AH, Wilkerson MJ, and **Ganta RR.** ***Ticks and Tick-borne Diseases***, 2021, 12, 101721. https://doi.org/10.1016/j.ttbdis.2021.101727
4. Development of a multiplex PCR and magnetic DNA capture assay for detecting six species pathogens of the genera *Anaplasma* and *Ehrlichia* in canine, bovine, caprine and ovine blood samples from Grenada, West Indies. Sharma B, **Ganta RR**, Stone D, Alhassan A, Lanza-Perea M, Belmar VM, Karasek I, Cooksey K, Butler CM, Gibson K, Wilkerson MJ. ***Pathogens*** 2021, 10, 192. https://doi.org/10.3390/pathogens10020192
5. Protein and DNA biosynthesis demonstrated in host cell-free phagosomes *containing Anaplasma phagocytophilum* or *Ehrlichia chaffeensis* in axenic media. Zhang Y, Chen L, Kondethimmanahalli C, Liu H, and **Ganta RR**. ***Infect. Immun.*** 2020, 89:e00638-20 (https://iai.asm.org/content/89/4/e00638-20)
6. Predicting the potential distribution of *Amblyomma americanum* (Acari: Ixodidae) infestation in New Zealand, using maximum entropy-based ecological niche modelling. Raghavan RK, Heath ACG, Lawrence KE, **Ganta RR**, Peterson AT, Pomroy WE. ***Exp. Appl. Acarol.*** 2020, 80:227-245.(https://link.springer.com/article/10.1007/s10493-019-00460-7)

E. ABSTRACTS:

None

F. PATENTS:

Attenuated vaccines to protect against tick-borne *Ehrlichia* species infections. U.S. Patent No. 10,434,161 issued on Oct. 8, 2019. Inventors: **Ganta RR,** Cheng C, Nair A, Jaworski D and Ganta S.

Attenuated vaccines to protect against tick-borne *Ehrlichia* species infections. U.S. Patent No.: 10,751,401; Issued date: August 25, 2020. Inventors: **Ganta RR,** Cheng C, Nair A, Jaworski D and Ganta S.

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C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Non-Compliant	Raghavan RK, Heath ACG, Lawrence KE, Ganta RR, Peterson AT, Pomroy WE. Predicting the potential distribution of Amblyomma americanum (Acari: Ixodidae) infestation in New Zealand, using maximum entropy-based ecological niche modelling. Experimental & applied acarology. 2020 February;80(2):227-245. PubMed PMID: 31965414; DOI: 10.1007/s10493-019-00460-7.
Complete	Wang Y, Nair ADS, Alhassan A, Jaworski DC, Liu H, Trinkl K, Hove P, Ganta CK, Burkhardt N, Munderloh UG, Ganta RR. Multiple Ehrlichia chaffeensis Genes Critical for Its Persistent Infection in a Vertebrate Host Are Identified by Random Mutagenesis Coupled with *In Vivo* Infection Assessment. Infection and immunity. 2020 September 18;88(10). PubMed PMID: 32747600; PubMed Central PMCID: PMC7504954; DOI: 10.1128/IAI.00316-20.
PMC Journal - In process	Zhang Y, Chen L, Kondethimmanahalli C, Ganta RR. Protein and DNA Biosynthesis Demonstrated in Host Cell-Free Phagosomes Containing Anaplasma phagocytophilum or Ehrlichia chaffeensis in Axenic Media. Infection and immunity. 2021 March 17;89(4):e00638-20.
Complete	Sharma B, R Ganta R, Stone D, Alhassan A, Lanza-Perea M, Matthew Belmar V, Karasek I, Cooksey E, M Butler C, Gibson K, J Wilkerson M. Development of a Multiplex PCR and Magnetic DNA Capture Assay for Detecting Six Species Pathogens of the Genera *Anaplasma* and *Ehrlichia* in Canine, Bovine, Caprine and Ovine Blood Samples from Grenada, West Indies. Pathogens (Basel, Switzerland). 2021 February 10;10(2). PubMed PMID: 33578784; PubMed Central PMCID: PMC7916465; DOI: 10.3390/pathogens10020192.
Non-Compliant	Alhassan A, Hove P, Sharma B, Matthew-Belmar V, Karasek I, Lanza-Perea M, Werners AH, Wilkerson MJ, Ganta RR. Molecular detection and characterization of Anaplasma platys and Ehrlichia canis in dogs from the Caribbean. Ticks and tick-borne diseases. 2021 April 9;12(4):101727. PubMed PMID: 33865177; DOI: 10.1016/j.ttbdis.2021.101727.
PMC Journal - In process	Unpublished

Non-compliant Publications Previously Reported for this Project

Public Access Compliance	Citation
Non-Compliant	Kim D, Jaworski DC, Cheng C, Nair AD, Ganta RR, Herndon N, Brown S, Park Y. The transcriptome of the lone star tick, Amblyomma americanum, reveals molecular changes in response to infection with the pathogen, Ehrlichia chaffeensis. Journal of Asia-Pacific Entomology. 2018 September;21(3):852-863. DOI: 10.1016/j.aspen.2018.05.009.
Non-Compliant	Wilkerson MJ, Black KE, Lanza-Perea M, Sharma B, Gibson K, Stone DM, George A, Nair AD, Ganta RR. Initial development and preliminary evaluation of a multiplex bead assay to detect

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	antibodies to Ehrlichia canis, Anaplasma platys, and Ehrlichia chaffeensis outer membrane peptides in naturally infected dogs from Grenada, West Indies. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 2017 January;29(1):109-114. PubMed PMID: 27852813; DOI: 10.1177/1040638716671979.
Non-Compliant	Cheng C, Ganta RR. Laboratory maintenance of Ehrlichia chaffeensis and Ehrlichia canis and recovery of organisms for molecular biology and proteomics studies. Current protocols in microbiology. 2008 May;Chapter 3:Unit 3A.1. PubMed PMID: 18770537; DOI: 10.1002/9780471729259.mc03a01s9.
Non-Compliant	Reinbold JB, Coetzee JF, Hollis LC, Nickell JS, Riegel C, Olson KC, Ganta RR. The efficacy of three chlortetracycline regimens in the treatment of persistent Anaplasma marginale infection. Veterinary microbiology. 2010 September 28;145(1-2):69-75. PubMed PMID: 20346598; DOI: 10.1016/j.vetmic.2010.02.031.
Non-Compliant	Cheng C, Sirigireddy KR, Ganta RR. Isolation and molecular detection of Ehrlichia from vertebrate animals. Current protocols in microbiology. 2008 May;Chapter 3:Unit 3A.3. PubMed PMID: 18770538; DOI: 10.1002/9780471729259.mc03a03s9.
Non-Compliant	Reinbold JB, Coetzee JF, Hollis LC, Nickell JS, Riegel CM, Christopher JA, Ganta RR. Comparison of iatrogenic transmission of Anaplasma marginale in Holstein steers via needle and needle-free injection techniques. American journal of veterinary research. 2010 October;71(10):1178-88. PubMed PMID: 20919904; DOI: 10.2460/ajvr.71.10.1178.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? Yes

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

Obtained via FOIA by White Coat Waste Project

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	GANTA, ROMAN R.	BS,MS,PHD	PD/PI	EFFORT					NA
	N	Redacted by agreement	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N		PHD	Staff scientist (Doctoral level)						NA
	N		PHD	Co-Investigator						NA
	N		PHD,BS,MS	Co-Investigator						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

Obtained via FOIA by White Coat Waste Project

D.2.d New Other Significant Contributors **Are there, or will there be, new other significant contributors?** No
D.2.e Multi-PI (MPI) Leadership Plan **Will there be a change in the MPI Leadership Plan for the next budget period?** NA

Obtained via FOIA by White Coat Waste Project

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

Obtained via FOIA by White Coat Waste Project

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subject

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

Obtained via FOIA by White Coat Waste Project

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name	DUNS	Congressional District	Address
Primary: Kansas State University	929773554	KS-001	Kansas State University 2 Fairchild Hall Manhattan, KS 665061103
KANSAS STATE UNIVERSITY	929773554		KANSAS STATE UNIVERSITY 2 FAIRCHILD HALL, 1601 VATTIER STREET

Obtained via FOIA by White Coat Waste Project

			MANHATTAN, KS 665062504

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period? No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Obtained via FOIA by White Coat Waste Project

From: Paul Lowe
To: Qualls, Mildred (NIH/NIAID) [E]
Cc: Roman Ganta; Carmen Garcia; Bailey Starns
Subject: Grant Number: 2R56AI070908 - 06 PI Name: GANTA, ROMAN R.
Date: Friday, August 02, 2013 4:02:49 PM
Attachments: Ganta Additional Info.pdf

Dear Dr. Qualls,

Below please find the responses to your questions:

Other Support provided for Dr. Munderloh indicates over commitment. Please let me know what will be done in order for Dr. Munderloh's effort not to exceed EFFORT

This was an oversight, Dr. Munderloh's revised Other Support document is attached.

- Confirm Kansas State University's most current rate agreement is dated 9/10/2012 with a rate of 50% or provide a copy of the latest agreement.
  Attached is a copy of KSU's latest rate agreement dated 9/10/2012 with a rate of 50% MTDC.
- Provide a copy of Oklahoma State University's rate agreement.
  Attached is a copy of OSU's latest rate agreement.
- Explain why a copy of Strategic Diagnostics, Inc's OLAW assurance letter was provided.
  Dr. Ganta will custom order antibodies using this company. He was not sure if NIH would require an assurance letter from the company, therefore the information was included in the proposal.

Please contact us if additional information is required.

Thank you.

Sincerely,

Paul R. Lowe
Assistant Vice President for Research

From: "Mildred Qualls (NIH/NIAID) [E]" <MMartinez@niaid.nih.gov>
To: rganta@ksu.edu, research@ksu.edu
Sent: Thursday, August 1, 2013 1:24:46 PM
Subject: Grant Number: 2R56AI070908 - 06 PI Name: GANTA, ROMAN R.

Dear Grantee,
I'm in the process of reviewing the grant mentioned above for possible funding and will require the following additional information in order to complete my review:
Other Support provided for Dr. Munderloh indicates over commitment. Please let me know what will be done in order for Dr. Munderloh's effort not to exceed EFFORT

- Confirm Kansas State University's most current rate agreement is dated 9/10/2012 with a rate of 50% or provide a copy of the latest agreement.

Obtained via FOIA by White Coat Waste Project

MUNDERLOH, U

Active

5R01AI042792 (Munderloh U) 05/01/2010 – 04/30/2015 Person Mos.: EFFORT
NIH (NIAID) $575,761
Tick-Mammal Interface of Human Granulocytic Anaplasmosis

Major goals are 1) Genomic comparison of non-human-pathogenic *Ap*-variants from an enzootic focus and isolates from human patients (*Ap*-ha); 2: Transcription analysis of *Anaplasma phagocytophilum* in human, tick, and mouse models; 3) Development of a transposon-mediated mutant library of *Ap* with altered phenotypes; 4) Integration of transcription data and mutagenesis data from aims 1, 2 and 3.

1R01AI081690 (Munderloh U) 01/15/2010 – 12/31/2014 Person Mos.: EFFORT
NIH (NIAID) $371,729
The Role of Plasmids in Rickettsia Biology

Major goals are 1) Comparative Sequence Analysis of Rickettsial Plasmids to understand the distribution and phylogeny of plasmids among *Rickettsia* spp. associated with blood-feeding arthropods that span the observed range of host adaptation and virulence within the genus. 2) Rickettsial Plasmid Curing and Conjugative Transfer to define the role of rickettsial plasmids in host adaptation/virulence, rickettsial genetic diversity and horizontal gene transfer.

2R01AI048424-07 (Munderloh U) 12/01/2003 - 11/30/2013 Person Mos.: EFFORT
NIH/NIAID $311,608
Development of Paratransgenic Ticks for Disease Control

Major goals: Aim 1. Genomics and functional genomics of a rickettsial candidate for paratransgenesis: a) Shotgun-sequence the *Rickettsia peacockii* genome. b) Analyze global gene expression analysis of *R. peacockii* using whole genome tiling arrays for comparison with *Rickettsia rickettsii* and other rickettsiae. Aim 2. Novel strategies for the genetic manipulation of rickettsial symbionts: a) Construct a rickettsial shuttle vector using rickettsial plasmid sequences as a guide. b) Optimize *Himar1* transposase system for rickettsiae. Aim 3. In vivo and in vitro biology of transgenic rickettsial symbionts: Real-time imaging of fluorescent protein expressing rickettsiae in live vector ticks and cultured tick cells.

1R13AI108235-01 (Munderloh) 06/01/2013 – 05/31/2014 Person Mos.: EFFORT
NIH (NIAID/IRD) $15,500
Annual American Society for Rickettsiology (ASR) Workshop

Major goal: To hold a workshop within the framework of the annual ASR meeting that addresses the central theme of “Regulation of Gene Expression in Rickettsiae.” This broadly includes molecular determinants of successful colonization of the arthropod vector and the mammalian host, defensive responses of arthropods and mammals, and genetic manipulation of pathogens to identify the regulatory mechanisms that coordinate this interplay. The workshop comprises oral presentations by key speakers selected from senior faculty, junior faculty, postdoctoral scientists and students, as well as posters. Travel support was provided for junior faculty, postdoctoral scientists and students, especially from underrepresented groups and resource-poor countries.

Pending:

Pending Support

Obtained via FOIA by White Coat Waste Project

Pending Support

Pending Support

Pending Support

OVERLAP

None

Obtained via FOIA by White Coat Waste Project

COLLEGES AND UNIVERSITIES RATE AGREEMENT

EIN: 1480771751A1

ORGANIZATION:
Kansas State University
102 Anderson Hall
Manhattan, KS 66506-0108

DATE:09/10/2012

FILING REF.: The preceding agreement was dated 08/12/2008

The rates approved in this agreement are for use on grants, contracts and other agreements with the Federal Government, subject to the conditions in Section III.

SECTION I: Facilities And Administrative Cost Rates

RATE TYPES: FIXED FINAL PROV. (PROVISIONAL) PRED. (PREDETERMINED)

	EFFECTIVE PERIOD				
TYPE	FROM	TO	RATE(%)	LOCATION	APPLICABLE TO
PRED.	07/01/2012	06/30/2016	50.00	On Campus	Organized Research
PRED.	07/01/2012	06/30/2016	50.00	On Campus	Instruction
PRED.	07/01/2012	06/30/2016	36.00	On Campus	Branch Station
PRED.	07/01/2012	06/30/2016	43.00	On Campus	AGR
PRED.	07/01/2012	06/30/2016	35.00	On Campus	OSA
PRED.	07/01/2012	06/30/2016	26.00	Off Campus	All Programs
PROV.	07/01/2016	Until Amended			Use same rates and conditions as those cited for fiscal year ending June 30, 2016.

*BASE

Obtained via FOIA by White Coat Waste Project

Modified total direct costs, consisting of all salaries and wages, fringe benefits, materials, supplies, services, travel and subgrants and subcontracts up to the first $25,000 of each subgrant or subcontract (regardless of the period covered by the subgrant or subcontract). Modified total direct costs shall exclude equipment, capital expenditures, charges for patient care, student tuition remission, rental costs of off-site facilities, scholarships, and fellowships as well as the portion of each subgrant and subcontract in excess of $25,000.

Obtained via FOIA by White Coat Waste Project

SECTION II: SPECIAL REMARKS

TREATMENT OF FRINGE BENEFITS:

The fringe benefits are specifically identified to each employee and are charged individually as direct costs. The directly claimed fringe benefits are listed below.

TREATMENT OF PAID ABSENCES

Vacation, holiday, sick leave pay and other paid absences are included in salaries and wages and are claimed on grants, contracts and other agreements as part of the normal cost for salaries and wages. Separate claims are not made for the cost of these paid absences.

OFF-CAMPUS DEFINITION: For all activities performed in facilities not owned by the institution and to which rent is directly allocated to the project(s) the off-campus rate will apply. Grants or contracts will not be subject to more than one F&A cost rate. If more than 50% of a project is performed off-campus, the off-campus rate will apply to the entire project.

Equipment Definition -
Equipment means an article of nonexpendable, tangible personal property having a useful life of more than one year and an acquisition cost of $5,000 or more per unit.

FRINGE BENEFITS:

FICA
TIAA/CREF
Retirement
Disability Insurance
Worker's Compensation
Life Insurance
Unemployment Insurance
Health Insurance
Dental Insurance
Severance Allowance

Obtained via FOIA by White Coat Waste Project

ORGANIZATION: Kansas State University

AGREEMENT DATE: 9/10/2012

SECTION III: GENERAL

A. LIMITATIONS:

The rates in this Agreement are subject to any statutory or administrative limitations and apply to a given grant, contract or other agreement only to the extent that funds are available. Acceptance of the rates is subject to the following conditions: (1) Only costs incurred by the organization were included in its facilities and administrative cost pools as finally accepted: such costs are legal obligations of the organization and are allowable under the governing cost principles; (2) The same costs that have been treated as facilities and administrative costs are not claimed as direct costs; (3) Similar types of costs have been accorded consistent accounting treatment; and (4) The information provided by the organization which was used to establish the rates is not later found to be materially incomplete or inaccurate by the Federal Government. In such situations the rate(s) would be subject to renegotiation at the discretion of the Federal Government.

B. ACCOUNTING CHANGES:

This Agreement is based on the accounting system purported by the organization to be in effect during the Agreement period. Changes to the method of accounting for costs which affect the amount of reimbursement resulting from the use of this Agreement require prior approval of the authorized representative of the cognizant agency. Such changes include, but are not limited to, changes in the charging of a particular type of cost from facilities and administrative to direct. Failure to obtain approval may result in cost disallowances.

C. FIXED RATES:

If a fixed rate is in this Agreement, it is based on an estimate of the costs for the period covered by the rate. When the actual costs for this period are determined, an adjustment will be made to a rate of a future year(s) to compensate for the difference between the costs used to establish the fixed rate and actual costs.

D. USE BY OTHER FEDERAL AGENCIES:

The rates in this Agreement were approved in accordance with the authority in Office of Management and Budget Circular A-21, and should be applied to grants, contracts and other agreements covered by this Circular, subject to any limitations in A above. The organization may provide copies of the Agreement to other Federal Agencies to give them early notification of the Agreement.

E. OTHER:

If any Federal contract, grant or other agreement is reimbursing facilities and administrative costs by a means other than the approved rate(s) in this Agreement, the organization should (1) credit such costs to the affected programs, and (2) apply the approved rate(s) to the appropriate base to identify the proper amount of facilities and administrative costs allocable to these programs.

BY THE INSTITUTION:

Kansas State University

(INSTITUTION)

Personal Info

(SIGNATURE)

Fran Willbrant

(NAME)

Assistant Vice President

(TITLE)

10-8-12

(DATE)

ON BEHALF OF THE FEDERAL GOVERNMENT:

DEPARTMENT OF HEALTH AND HUMAN SERVICES

(AGENCY)

Personal Info

(SIGNATURE)

Arif Karim

(NAME)

Director, Central States Field Office

(TITLE)

9/10/2012

(DATE) 7033

HHS REPRESENTATIVE: Denise Shirlee

Telephone: (214) 767-3261

Obtained via FOIA by White Coat Waste Project

COMPONENTS OF THE PUBLISHED FACILITIES & ADMINISTRATIVE COST RATE

INSTITUTION: Kansas State University

FY COVERED BY RATE: FY 2013 - FY 2016

RATE TYPE:	ORGANIZED RESEARCH	INSTRUCTION	BRANCH STATION	AGRICULTURE	OTHER SPONSORED ACTIVITIES	OFF CAMPUS
	On-Camp	On-Camp	On-Camp	On-Camp	On-Camp	Off-Camp
	FY 2013 - 2016	FY 2013 - 2016	FY 2013 - 2016	FY 2013 - 2016	FY 2013 - 2016	FY 2013 - 2016
RATE COMPONENTS:						
Building Depreciation	4.2	2.5	1.0	2.0	1.0	
Equipment Depreciation	3.0	0.9	3.0	1.7	0.5	
Interest	1.1	0.4	0.0	0.2	0.0	
Operations & Maintenance	14.7	9.3	5.0	12.1	6.5	
Library	1.0	10.9	1.0	1.0	1.0	
Administrative Component	26.0	26.0	26.0	26.0	26.0	26.0
F&A Rate	50.0	50.0	36.0	43.0	35.0	26.0

CONCURRENCE:

Kansas State University
(Institution)

Personal Info

(Signature)

Fran Willbrant
(Name)

Assistant Vice President
(Title)

10-8-12
(Date)

Obtained via FOIA by White Coat Waste Project

DEPARTMENT OF THE NAVY
OFFICE OF NAVAL RESEARCH
875 NORTH RANDOLPH STREET
SUITE 1425
ARLINGTON, VA 22203-1995

IN REPLY REFER TO:

Agreement Date: August 2, 2012
Superseded Agreement Dated: June 29, 2012

NEGOTIATION AGREEMENT

INSTITUTION: OKLAHOMA STATE UNIVERSITY
STILLWATER, OKLAHOMA 74078-1028

The Facilities and Administrative (F&A) rates contained herein are for use on grants, contracts and/or other agreements issued or awarded to Oklahoma State University by all Federal Agencies of the United States of America, in accordance with the cost principles mandated by 2 CFR 220. These rates shall be used for forward pricing and billing purposes at Oklahoma State University for Fiscal Years 2013-2016. This rate agreement supersedes all previous rate agreements/determinations for Fiscal Years 2013-2016.

SECTION I: RATES - TYPE: PREDETERMINED (Pred)
PROVISIONAL (Prov)

TYPE	FROM	TO	RATE On-Campus	RATE Off Campus	BASE	APPLICABLE TO
Stillwater Campus*						
Pred	7/1/12	6/30/15	74.6%	45.8%	(a)	Instruction (1)
Prov	7/1/16	Until Amended	74.6%	45.8%	(a)	Instruction (1)
Pred	7/1/12	6/30/15	54.8%	26.0%	(a)	Instruction (2)
Prov	7/1/16	Until Amended	54.8%	26.0%	(a)	Instruction (2)
Pred	7/1/12	6/30/15	45.8%	25.3%	(a)	Research (1)
Prov	7/1/16	Until Amended	45.8%	25.3%	(a)	Research (1)
Pred	7/1/12	6/30/15	45.8%	25.3%	(a)	Research (2)
Prov	7/1/16	Until Amended	45.8%	25.3%	(a)	Research (2)
Pred	7/1/12	6/30/15	48.4%	35.7%	(a)	Extension (1)
Prov	7/1/16	Until Amended	48.4%	35.7%	(a)	Extension (1)
Pred	7/1/12	6/30/15	38.7%	26.0%	(a)	Extension (2)
Prov	7/1/16	Until Amended	38.7%	26.0%	(a)	Extension (2)

*Rates also applicable to Oklahoma State University Center for Innovation and Economic Development, Inc. (CIED).

RECEIVED
SEP 06 2012
GRANTS & CONTRACTS
FIN. ADMIN.

Obtained via FOIA by White Coat Waste Project

TYPE	FROM	TO	RATE On-Campus	RATE Off Campus	BASE	APPLICABLE TO
Okmulgee Campus						
Pred	7/1/12	6/30/15	64.9%	37.5%	(a)	All Programs (1)
Prov	7/1/16	Until Amended	64.9%	37.5%	(a)	All Programs (1)
Pred	7/1/12	6/30/15	53.5%	26.0%	(a)	All Programs (2)
Prov	7/1/16	Until Amended	53.5%	26.0%	(a)	All Programs (2)
Oklahoma City Campus						
Pred	7/1/12	6/30/15	65.3%	37.3%	(a)	All Programs (1)
Prov	7/1/16	Until Amended	65.3%	37.3%	(a)	All Programs (1)
Pred	7/1/12	6/30/15	54.0%	26.0%	(a)	All Programs (2)
Prov	7/1/16	Until Amended	54.0%	26.0%	(a)	All Programs (2)
Center for Health Sciences						
Pred	7/1/12	6/30/15	27.6%	17.3%	(a)	All Programs (1)
Prov	7/1/16	Until Amended	27.6%	17.3%	(a)	All Programs (1)
Pred	7/1/12	6/30/15	27.6%	17.3%	(a)	All Programs (2)
Prov	7/1/16	Until Amended	27.6%	17.3%	(a)	All Programs (2)
Tulsa Campus						
Pred	7/1/12	6/30/15	92.4%	52.8%	(a)	All Programs (1)
Prov	7/1/16	Until Amended	92.4%	52.8%	(a)	All Programs (1)
Pred	7/1/12	6/30/15	65.6%	26.0%	(a)	All Programs (2)
Prov	7/1/16	Until Amended	65.6%	26.0%	(a)	All Programs (2)

DISTRIBUTION BASE

(a) Modified total direct costs, consisting of all salaries and wages, fringe benefits, materials and supplies, services, travel, and subgrants and subcontracts up to the first $25,000 of each subgrant or subcontract (regardless of the period covered by the subgrant or subcontract). Equipment, capital expenditures, charges for patient care and tuition remission, rental costs, scholarships, and fellowships as well as the portion of each subgrant and subcontract in excess of $25,000 shall be excluded from modified total direct costs.

APPLICABLE TO:

(1) Applies to only DOD Contracts awarded on or after November 30, 1993 in accordance with and under the authority of DFARS 231.303(1). See Section II, Paragraph F hereof.

(2) Applies to all Non-DOD Instruments, all DOD Grants and to DOD Contracts awarded before November 30, 1993. See Section II, Paragraph F hereof.

Obtained via FOIA by White Coat Waste Project

SECTION II: GENERAL TERMS AND CONDITIONS

A. **LIMITATIONS**: Use of the rates set forth under Section I is subject to any statutory or administrative limitations and is applicable to a given grant, contract or other agreement only to the extent that funds are available and consistent with any and all limitations of cost clauses or provisions, if any, contained therein. Acceptance of any or all of the rates agreed to herein is predicated upon all the following conditions: (1) that no costs other than those incurred by the grantee/contractor were included in its indirect cost pool as finally accepted and that all such costs are legal obligations of the grantee/contractor and allowable under governing cost principles; (2) that the same costs that have been treated as indirect costs are not claimed as direct costs; (3) that similar types of costs, in like circumstances, have been accorded consistent accounting treatment; (4) that the information provided by the contractor/grantee, which was used as the basis for the acceptance of the rates agreed to herein and expressly relied upon by the Government in negotiating the said rates, is not subsequently found to be materially incomplete or inaccurate.

B. **ACCOUNTING CHANGES**: The rates contained in Section I of this agreement are based on the accounting system in effect at the time this agreement was negotiated. Changes to the method(s) of accounting for costs, which affects the amount of reimbursement resulting from the use of these rates, require the written approval of the authorized representative of the cognizant negotiating agency for the Government prior to implementation of any such changes. Such changes include but are not limited to changes in the charging of a particular type of cost from indirect to direct. Failure to obtain such approval may result in subsequent cost disallowances.

C. **PREDETERMINED RATES:** The predetermined rates contained in this agreement are not subject to adjustment in accordance with the provisions of 2 CFR 220, subject to the limitations contained in Part A of this section.

D. **PROVISIONAL RATES**: The provisional rates contained in this agreement are subject to unilateral amendment by the Government or bilateral amendment by the contracting parties at any time.

E. **USE BY OTHER FEDERAL AGENCIES**: The rates set forth in Section I hereof were negotiated in accordance with and under the authority set forth in 2 CFR 220. Accordingly, such rates shall be applied to the extent provided in such regulations to grants, contracts and other transactions to which 2 CFR 220 is applicable, subject to any limitations in part A of this section. Copies of this document may be provided by either party to other Federal agencies which have or intend to issue or award grants and contracts using these rates or to otherwise provide such agencies with documentary notice of this agreement and its terms and conditions.

Obtained via FOIA by White Coat Waste Project

F. **APPLICATION OF INDIRECT COST RATES TO DOD CONTRACTS/SUBCONTRACTS:**

In accordance with DFARS 231.303, no limitation (unless waived by the institution) may be placed on the reimbursement of otherwise allowable indirect costs incurred by an institution of higher education under a DOD contract awarded on or after November 30, 1993, unless the same limitation is applied uniformly to all other organizations performing similar work. It has been determined by the Department of Defense that such limitation is not being uniformly applied. Accordingly, the rates cited (1) of Section I, as explained under the title, "APPLICABLE TO" do not reflect the application of the 26% limitation on administrative indirect costs imposed by 2 CFR 220, whereas (2) do so.

Accepted:

FOR OKLAHOMA STATE UNIVERSITY:

Personal Info

Kathy Kamm Elliott
Associate Vice President and Controller

8/8/12
Date

FOR THE GOVERNMENT:

Personal Info

David F. Godfrey
Contracting Officer

8/28/12
Date

For information concerning this agreement contact:

DAVID F. GODFREY, Contracting Officer
Office of Naval Research
875 North Randolph Street
Arlington, VA 22203-1995

Phone: (703) 696-2586
E-mail: david.f.godfrey@navy.mil

Obtained via FOIA by White Coat Waste Project

- Provide a copy of Oklahoma State University's rate agreement.
- Explain why a copy of Strategic Diagnostics, Inc's OLAW assurance letter was provided.

Please submit the following information via email from an authorized business official by Monday 8/5/2013.

Thanks,
Mildred

Mildred J. Qualls

NIH National Institute of Allergy and Infectious Diseases

Grants Management Specialist
Room 2125
6700B Rockledge Drive, MSC 7614
Bethesda, MD 20892 (Regular Mail)
Bethesda, MD 20817 (Express Mail)
Phone: (301) 402-6611
FAX: (301) 493-0597
Email: mq20b@nih.gov

Disclaimer: The information in this e-mail and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the original intended recipient. If you have received this e-mail in error please inform the sender and delete it from your mailbox or any other storage devices. National Institute of Allergy and Infectious Diseases shall not accept liability for any statements made that are sender's own and not expressly made on behalf of the NIAID by one of its representatives.

Obtained via FOIA by White Coat Waste Project

Just In Time Report

Report submitted on : 08/01/2013 12:12 PM

IRB Confirmation:	No IRB Certification was required
Human Subjects Assurance Number:	No Human Subjects Assurance was required
Human Subjects Education:	No Human Subjects Education was provided
IACUC Confirmation:	01/29/2013

Obtained via FOIA by White Coat Waste Project

GANTA, R

ACTIVE THIS GRANT T5

R01 AI070908A (Ganta R) 12/15/07-11/30/13 Person Mos.
NIH NIAID $309,417 EFFORT
Vector and host contributions to the regulation of *E. chaffeensis* gene expression

The major goals of this project are to evaluate the contributions of vector and host cell environment in altering the pathogen gene expression.

1P30 GM103326-01A1 (Govind R) 12/03/12-07/31/13 Person Mos.
University of Kansas Medical Center Research Institute $60,000 EFFORT
(NIH Flowthrough)

Role of TcdR, the alternate sigma factor in Clostridium difficile virulence
Role on the project for Ganta: Mentor for KSU Subaward PI, Govind.

5 P20 GM103418-13 (Govind, R) 05/01/13-04/30/14 Person Mos.
University of Kansas Medical Center Research Institute $15,000 EFFORT
KINBRE Major Starter Grant Mentor – Roman Ganta (Ganta's portion)
(NIH Flowthrough)

The major goal of this Dr. Govind's project is to determine the toxin secretion mechanism in *Clostridium difficile*
Role on the project for Ganta: Mentor for KSU Subaward PI, Govind.

Private Source Ganta R) 09/01/12-08/31/13 Person Mos.
$106,017 EFFORT

Clinical and diagnostic assessment of Ehrlichia and Anaplasma species infections in dogs
The major goal of this project is to conduct experimental infections in dogs with five closely related rickettsial pathogens; *Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia ewingii, Anaplasma phagocytophilum* and *Anaplasma platys*.

PENDING THIS PROJECT

Pending Support

Pending Support

Obtained via FOIA by White Coat Waste Project

Pending Support

Pending Support

OVERLAP
None

JAWORSKI, D

CURRENT
None

PENDING

Pending Support

OVERLAP
None

MUNDERLOH, U

Active

5R01AI042792 (Munderloh U) 05/01/2010 – 04/30/2015 Person Mos. EFFORT
NIH (NIAID) $575,761
Tick-Mammal Interface of Human Granulocytic Anaplasmosis

Major goals are 1) Genomic comparison of non-human-pathogenic *Ap*-variants from an enzootic focus and isolates from human patients (*Ap*-ha); 2: Transcription analysis of *Anaplasma phagocytophilum* in human, tick, and mouse models; 3) Development of a transposon-mediated mutant library of *Ap* with altered phenotypes; 4) Integration of transcription data and mutagenesis data from aims 1, 2 and 3.

1R01AI081690 (Munderloh U) 01/15/2010 – 12/31/2014 Person Mos.: EFFORT
NIH (NIAID) $371,729
The Role of Plasmids in Rickettsia Biology

Major goals are 1) Comparative Sequence Analysis of Rickettsial Plasmids to understand the distribution and phylogeny of plasmids among *Rickettsia* spp. associated with blood-feeding arthropods that span the observed range of host adaptation and virulence within the genus. 2) Rickettsial Plasmid Curing and Conjugative Transfer to define the role of rickettsial plasmids in host adaptation/virulence, rickettsial genetic diversity and horizontal gene transfer.

2R01AI048424-07 (Munderloh U) 12/01/2003 - 11/30/2013 Person Mos. EFFORT
NIH/NIAID $311,608
Development of Paratransgenic Ticks for Disease Control

Obtained via FOIA by White Coat Waste Project

Major goals: Aim 1. Genomics and functional genomics of a rickettsial candidate for paratransgenesis: a) Shotgun-sequence the *Rickettsia peacockii* genome. b) Analyze global gene expression analysis of *R. peacockii* using whole genome tiling arrays for comparison with *Rickettsia rickettsii* and other rickettsiae. Aim 2. Novel strategies for the genetic manipulation of rickettsial symbionts: a) Construct a rickettsial shuttle vector using rickettsial plasmid sequences as a guide. b) Optimize *Himar1* transposase system for rickettsiae. Aim 3. In vivo and in vitro biology of transgenic rickettsial symbionts: Real-time imaging of fluorescent protein expressing rickettsiae in live vector ticks and cultured tick cells.

1R13AI108235-01 (Munderloh) 06/01/2013 – 05/31/2014 Person Mos.: EFFORT
NIH (NIAID/IRD) $15,500
Annual American Society for Rickettsiology (ASR) Workshop

Major goal: To hold a workshop within the framework of the annual ASR meeting that addresses the central theme of “Regulation of Gene Expression in Rickettsiae.” This broadly includes molecular determinants of successful colonization of the arthropod vector and the mammalian host, defensive responses of arthropods and mammals, and genetic manipulation of pathogens to identify the regulatory mechanisms that coordinate this interplay. The workshop comprises oral presentations by key speakers selected from senior faculty, junior faculty, postdoctoral scientists and students, as well as posters. Travel support was provided for junior faculty, postdoctoral scientists and students, especially from underrepresented groups and resource-poor countries.

Pending:

Pending Support

Pending Support

Pending Support

Obtained via FOIA by White Coat Waste Project

Pending Support

OVERLAP

None

Obtained via FOIA by White Coat Waste Project

Oklahoma State University
Institutional Animal Care and Use Committee (IACUC)

Protocol Expires: 1/28/2016

Date : Tuesday, January 29, 2013 Animal Care and Use Protocol (ACUP) No : AG1219

Proposal Title: Vector and host contributions to the regulation of E. chaffeensis expression

Principal Investigator:

Deborah Jaworski
Entomology and Plant Pathology
127 NRC
Campus

Reviewed and Processed as: Full Committee

Approval Status Recommended by Reviewer(s) : Approved

The revised protocol is approved. You are approved to use a maximum of 63 deer for the next three years.

Signatures :

Personal Info

Charlotte Ownby, IACUC Chair

Tuesday, January 29, 2013
Date

cc: Department Head, Entomology and Plant Pathology
Director, Animal Resources

Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications to the research project, course, or testing procedure must be submitted for review and approval by the IACUC, prior to initiating any changes. Modifications do not affect the original approval period. Approved projects are subject to monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.

Obtained via FOIA by White Coat Waste Project

Division of Agricultural Sciences and Natural Resources

Department of Entomology & Plant Pathology
127 Noble Research Center
Stillwater, OK 74078
405.744.5643 Fax: 405.744.6039

Dr. Deborah Jaworski June 4, 2013

Dear Deborah,

This letter is sent to inform you that your request for appointment within our department at the level of Adjunct Assistant Professor has been reviewed by our RPT committee and voted on by the faculty. The results of that review and vote indicate a desire on our part to continue that relationship. Please understand that this appointment is effective for a four year period, starting June 1, 2013 and that it will allow you to complete the termination report of your Hatch project and continue to serve on your graduate student's committee. I will also forward your materials to Group I for inclusion as a Graduate Faculty member. Thank you for your time and efforts in completing this process. Please feel free to contact me if you have questions concerning your appointment or involvement with our department.

Sincerely,

Personal Info

Phil Mulder
Professor and Head
Entomology and Plant Pathology
127 Noble Research Center
Oklahoma State University
Stillwater, OK 74078-3033
Phone: 405-744-9413
Email: phil.mulder@okstate.edu

Obtained via FOIA by White Coat Waste Project

PI Application Brief History

Accession #: 3543568
PI : GANTA, ROMAN R.
ORG: KANSAS STATE UNIVERSITY

RFA/PA #: PA11-260
DEG: PHD, MS, BS, MOTH
IRG Member: Y

Process Stage:
Received Date: 11/19/2012

MULTIPLE PIs
NONE

ORGANIZATION

NO UNFORWARDED ARA DATA

IRG:**RG BM-1** Grant #:**1 R21 AI050785-01 A1** Dual: Former #: 1R01AI050785-01A1 Acc.#:779421 Council: **05/2002**

ORG: KANSAS STATE UNIVERSITY MANHATTAN IPF: 4202801 EIN: 1480771751A1 Appl. # :6542107

PI: GANTA, ROMAN R DEG: PHD Phone: (785) 532-4612

Title: **Cellular Immunity Against Ehrlichia chaffeensis** RFA/PA: Fax: (785) 532-4851

Proj. Date: 08/01/2002 - 07/31/2004 AIDS: Human: Evalua Animal: Evaluati E-Mail: RGANTA@VET.KSU.EDU

Bud. Date: 08/01/2002 - 07/31/2004 Future Yr: Gender: Minority: Summary Stat: Y CA: 1

Award: $352,375 Direct: $250,000 Status: Awarded. Enc.: 02/10/2004 REF CD:1 Percentile: Evalu Pri Score: Evaluativ

PC: M46C PO: Perdue, Samuel S. Phone: 301-402-5083 E-Mail: sperdue@niaid.nih.gov

Unfunded Info

Unfunded Info

IRG:**RG ZRG1 BM-1(02) Q** Grant #:**5 R01 AI055052-05** Dual: Former #: 1R21AI050785-01A1 Acc.#: Council: **00/2007**

ORG: KANSAS STATE UNIVERSITY MANHATTAN IPF: 4202801 EIN: 1480771751A1 Appl. # :7169913

PI: GANTA, ROMAN R. DEG: PHD, MS, BS Phone: (785) 532-4612

Title: **Cellular Immunity Against Ehrlichia chaffeensis** RFA/PA: Fax: (785) 532-4851

Proj. Date: 09/30/2003 - 01/31/2009 AIDS: Human: Evaluat Animal: Evaluative Info E-Mail: RGANTA@VET.K-STATE.EDU

Bud. Date: 02/01/2007 - 01/31/2009 Future Yr: Gender: Minority: Summary Stat: N CA:

Award: $346,086 Direct: $237,045 Status: Awarded. Enc.: 07/16/2007 REF CD:1 Percentile: Evalua Pri Score: Evaluativ

PC: M46C PO: Perdue, Samuel S. Phone: 301-402-5083 E-Mail: sperdue@niaid.nih.gov

Obtained via FOIA by White Coat Waste Project

PI Application Brief History

Unfunded Info

Unfunded Info

Unfunded Info

Unfunded Info

Obtained via FOIA by White Coat Waste Project

PI Application Brief History

IRG:**AI ZAI1 MJH-M(M1)** Grant #:**1 R13 AI066872-01** Dual: Former #: Acc.#:992793 Council: **05/2005**

ORG: KANSAS STATE UNIVERSITY MANHATTAN IPF: 4202801 EIN: 1480771751A1 Appl. # :7000838

PI: GANTA, ROMAN R DEG: PHD, MS Phone: (785) 532-4612

Title: **American Society for Rickettsiology conference** RFA/PA: PAR03-176 Fax: (785) 532-4851

Proj. Date: 06/01/2005 - 05/31/2006 AIDS: N Human: Evaluati ve Animal: Evaluative Info E-Mail: RGANTA@VET.K-STATE'EDU

Bud. Date: 06/01/2005 - 05/31/2006 Future Yr: Gender: Minority: Summary Stat: Y CA: 1

Award: $15,000 Direct: $15,000 Status: Awarded. Enc.: 05/20/2005 REF CD: Percentile: Pri Score: Evaluative Info

PC: M73F B PO: Garges, Susan Phone: 301 496-4550 E-Mail: sgarges@mail.nih.gov

Unfunded Info

Unfunded Info

Unfunded Info

Obtained via FOIA by White Coat Waste Project

PI Application Brief History

IRG:**RG ZRG1 IDM-B(02) M** Grant #:**5 R01 AI070908-05** Dual: Former #: Acc.#: Council: **00/2012**

ORG: KANSAS STATE UNIVERSITY Manhattan IPF: 4202801 EIN: 1480771751A1 Appl. # :8196905

PI: GANTA, ROMAN R. DEG: PHD, MS, BS Phone: (785) 532-4612

Title: **Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression** RFA/PA: PA07-070 Fax: (785) 532-4851

Proj. Date: 12/15/2007 - 11/30/2013 AIDS: N Human: Evaluativ e Animal: Evaluative E-Mail: RGANTA@VET.K-STATE.EDU

Bud. Date: 12/01/2011 - 11/30/2013 Future Yr: Gender: Minority: Summary Stat: N CA:

Award: $357,737 Direct: $245,025 Status: Awarded. Enc.: 05/24/2012 REF CD9 Percentile: Evalu ative Pri Score: Evaluative

PC: M46C PO: Mukhopadhyay, Suman Phone: 301-451-3032 E-Mail: mukhopadhyays@mail.nih.gov

IRG:**RG ZRG1 IDM-B(02) M(INT)** Grant #:**3 R01 AI070908-03 S1** Dual: Former #: Acc.#: Council: **00/2010**

ORG: KANSAS STATE UNIVERSITY Manhattan IPF: 4202801 EIN: 1480771751A1 Appl. # :8059879

PI: GANTA, ROMAN R. DEG: PHD Phone: (785) 532-4612

Title: **Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression** RFA/PA: PA07-070 Fax: (785) 532-4851

Proj. Date: 12/15/2007 - 11/30/2012 AIDS: N Human: Evaluati ve Animal: Evaluative Info E-Mail: RGANTA@VET.K-STATE.EDU

Bud. Date: 05/01/2010 - 11/30/2010 Future Yr: Gender: Minority: Summary Stat: N CA:

Award: $61,917 Direct: $61,917 Status: Awarded. Enc.: 04/15/2010 REF CD9 Percentile: Evalua ive Pri Score: Evaluative Info

PC: M46C PO: Perdue, Samuel S. Phone: 301-402-5083 E-Mail: sperdue@niaid.nih.gov

Unfunded Info

Unfunded Info

Obtained via FOIA by White Coat Waste Project

PI Application Brief History

IRG:**AI ZAI1 MJH-M(M1)** Grant #:**1 R13 AI071693-01** Dual: OD Former #: Acc.#:2916247 Council: **05/2006**

ORG: KANSAS STATE UNIVERSITY Manhattan IPF: 4202801 EIN: 1480771751A1 Appl. # :7162039

PI: GANTA, ROMAN R. DEG: PHD, MS, BS Phone: 785-532-4612

Title: **American Society for Rickettsiology Conference 2006** RFA/PA: PA06-041 Fax:

Proj. Date: 07/15/2006 - 06/30/2007 AIDS: N Human: Evaluative Info Animal: Evaluative Info E-Mail: rganta@vet.ksu.edu

Bud. Date: 07/15/2006 - 06/30/2007 Future Yr: Gender: Minority: Summary Stat: Y CA: 1

Award: $30,000 Direct: $30,000 Status: Awarded. Enc.: 07/07/2006 REF CD: Percentile: Pri Score: Evaluative Info

PC: M74B B PO: Garges, Susan Phone: 301 496-4550 E-Mail: sgarges@mail.nih.gov

IRG:**AI ZAI1 EB-M(M1)** Grant #:**1 R13 AI084375-01** Dual: OD Former #: Acc.#:3126840 Council: **05/2009**

ORG: KANSAS STATE UNIVERSITY Manhattan IPF: 4202801 EIN: 1480771751A1 Appl. # :7750247

PI: GANTA, ROMAN R. DEG: PHD, MS, BS Phone: 785-532-4612

Title: **American Society for Rickettsiology Conference 2009** RFA/PA: PA08-149 Fax: 785-532-4851

Proj. Date: 08/15/2009 - 07/31/2010 AIDS: N Human: Evaluative Info Animal: Evaluative Info E-Mail: rganta@vet.k-state.edu

Bud. Date: 08/15/2009 - 07/31/2010 Future Yr: Gender: Minority: Summary Stat: Y CA: 1

Award: $15,349 Direct: $15,349 Status: Awarded. Enc.: 09/04/2009 REF CD: Percentile: Pri Score: Evaluative Info

PC: M74B B PO: Mulach, Barbara L. Phone: 301-402-4199 E-Mail: bmulach@niaid.nih.gov

Unfunded Info

Pending Support

Obtained via FOIA by White Coat Waste Project

PI Application Brief History

Pending Support

Unfunded Info

Obtained via FOIA by White Coat Waste Project

From: Smith, Gregory (NIH/NIAID) [E]
To: "Carmen Garcia"
Cc: Bailey Starns
Subject: RE: R01 and R56 NOA Kansas State University, R. Ganta
Date: Wednesday, August 21, 2013 10:19:04 AM

Hi Carmen,

Although this grant falls in my assigned portfolio, other NIAID grants staff administered and awarded these. My apologies for their errors.

See my responses below.

Greg Smith
Lead Grants Management Specialist
DHHS/NIH/NIAID/GMP
Phone: (301) 402-5540
Fax: (301) 493-0597
gs225y@nih.gov

From: Carmen Garcia [mailto:carmen@ksu.edu]
Sent: Tuesday, August 13, 2013 10:45 AM
To: Smith, Gregory (NIH/NIAID) [E]
Cc: Bailey Starns
Subject: R01 and R56 NOA Kansas State University, R. Ganta

Hello Mr. Smith,

We have recieved 2 NOA's for Dr. Ganta's project, "Vector and Host Contributions to the Regulation of E. chaffeensis Gene Expression"
I have attached both NOA's here for your quick reference.

NOA 5R01 AI070908-05 Revised
*Page 1- Would it be possible to edit the end date to 08/11/13? Currently, the end date says 08/08/2013, this creates a gap of 3 days before the R56 starts on 08/12/13.
We want to avoid any problems for salaries/charges during those 3 missing days.
#1 I will revise to correct the R01 project period end dates to close the hiatus. THE REVISED NOA WITH CORRECTION WILL ISSUE 8/22/13

*Page 4- there is wording that says this award represents the final year- Should this be here given that the R56 was awarded?
#2 This term is required when a project segment ends. The R56 starts its own new 1 year competitive project segment apart from the R01 competitive project segment. This the final year of that segment and requires closeout with a FFR for years 1 thru 5. The Type 2 proposal submitted will serve as the final progress report for this 1 thru 5 project segment. NO ACTION TAKEN

*Page 5- REVISED AWARD: this sentence says R56 is effective 8/11/13, but the R56 NOA

Obtained via FOIA by White Coat Waste Project

says 08/12/13.
#3 This is a mistake and I will correct the term to reflect the R56 start date of 8/12/13. THE EVISED NOA WITH CORRECTION WILL ISSUE 8/22/13

NOA 2R56 AI070908-06
*Page 1- The start date of 08/12/13 is fine with KSU but we'd like the R01 to be changed (or vice versa) so that the termination/effective dates are consecutive.
This will be corrected by my action in #3 above. NO ACTION TAKEN

*Page 4- this NOA also has that this award represents the final year. Just checking to see if the wording is more appropriate here than on the R01. Not sure how that all works since we have 2 different mechanisms.
#4 This term is required when a project segment ends. The R56 1-year bridge is a 1 year project segment itself and thus is in its final year. NO ACTION TAKEN

Thank you for your help!

Carmen Garcia
Grant and Contract Administrator
Office of Research and Sponsored Programs
PreAward Services
2 Fairchild Hall
Manhattan, KS 66506-1103
PH (785) 532-6804 FX (785) 532-5944

Obtained via FOIA by White Coat Waste Project

From: Brooks, Lyndsay (NIH/NIAID) [E]
To: plowe@ksu.edu
Cc: Perdue, Samuel (NIH/NIAID) [E]; rganta@vet.k-state.edu
Subject: Grant Number: 5R01AI070908 - 11 PI Name: GANTA, ROMAN R.
Date: Wednesday, June 10, 2020 10:31:00 AM

Dear Mr. Lowe,

Thank you for your recent RPPR submission. Upon review of the document I have noticed that you have non-compliant publication(s). Below is a listing of the non-compliant publication(s):

- Cheng C, Ganta RR. Laboratory maintenance of Ehrlichia chaffeensis and Ehrlichia canis and recovery of organisms for molecular biology and proteomics studies. Current protocols in microbiology. 2008 May;Chapter 3:Unit 3A.1. PubMed PMID: 18770537: DOI: 10.1002/9780471729259.mc03a01s9.
- Cheng C, Sirigireddy KR, Ganta RR. Isolation and molecular detection of Ehrlichia from vertebrate animals. Current protocols in microbiology. 2008 May;Chapter 3:Unit 3A.3. PubMed PMID: 18770538; DOI: 10.1002/9780471729259.mc03a03s9.
- Reinbold JB, Coetzee JF, Hollis LC, Nickell JS, Riegel C, Olson KC, Ganta RR. The efficacy of three chlortetracycline regimens in the treatment of persistent Anaplasma marginale infection. Veterinary microbiology. 2010 September 28;145(1-2):69-75. PubMed PMID: 20346598; DOI: 10.1016/j.vetmic.2010.02.031.
- Reinbold JB, Coetzee JF, Hollis LC, Nickell JS, Riegel CM, Christopher JA, Ganta RR. Comparison of iatrogenic transmission of Anaplasma marginale in Holstein steers via needle and needle-free injection techniques. American journal of veterinary research. 2010 October;71(10):1178-88. PubMed PMID: 20919904; DOI: 10.2460/ajvr.71.10.1178.
- Wilkerson MJ, Black KE, Lanza-Perea M, Sharma B, Gibson K, Stone DM, George A, Nair AD, Ganta RR. Initial development and preliminary evaluation of a multiplex bead assay to detect antibodies to Ehrlichia canis, Anaplasma platys, and Ehrlichia chaffeensis outer membrane peptides in naturally infected dogs from Grenada, West Indies. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 2017 January;29(1):109-114. PubMed PMID: 27852813; DOI: 10.1177/1040638716671979.
- Kim D, Jaworski DC, Cheng C, Nair AD, Ganta RR, Herndon N, Brown S, Park Y. The transcriptome of the lone star tick, Amblyomma americanum, reveals molecular changes in response to infection with the pathogen, Ehrlichia chaffeensis. Journal of Asia-Pacific Entomology. 2018 September;21(3):852-863. DOI: 10.1016/j.aspen.2018.05.009.

Compliance with the NIH Public Access Policy is demonstrated through the myNCBI PDF report. Please submit this report to me through your business official. Please be sure that there are no "non-compliant" publications listed on the report when you submit it to me.

RPPR compliance enforcement has been suspended by NIH which will allow NIAID to issue this award for the RPPR showing one or more non-compliant publications. Please continue to work through the appropriate sources and systems to resolve the non-compliant publications listed in this email. It is expected that resolution of non-compliance for the listed publications occur prior to the end of the budget period of the upcoming NOA. NIAID will revisit any unresolved

Obtained via FOIA by White Coat Waste Project

publication issues when the next RPPR is submitted, if applicable. A term and condition to reflect the suspension of enforcement and the actions required by the Grantee has been placed on the NOA.

Sincerely,

Lyndsay Brooks
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
lyndsay.brooks@nih.gov

Obtained via FOIA by White Coat Waste Project

From:	Smith, Gregory (NIH/NIAID) [E]
To:	"rganta@ksu.edu"; "plowe@k-state.edu"
Cc:	Perdue, Samuel (NIH/NIAID) [E]; Smith, Gregory (NIH/NIAID) [E]
Subject:	URGENT JIT Update Requested RE: 2R01AI070908-06A1
Date:	Tuesday, July 22, 2014 4:31:53 PM

Dear Dr. Ganta and KSU Business Officials:

Congratulations! Your above referenced grant was selected for 4 years of discretionary funding. **The following JIT request does not guarantee your grant will be funded. Any official notification of funding can only be provided by a Notice of Grant Award. Be advised we are operating in our end of fiscal year processing so funding decisions must be made quickly.**

Your JIT originally submitted for this grant is greater than 120 days old and must be revised/update to be considered valid. The revised JIT should be submitted no later than end of business on July 25, 2014:

Please ensure your updated JIT includes the following:

- Complete Other Support for all active and pending projects for Dr. Roman R. Ganta
- Current IACUC approval date

As requested the JIT should be updated in the COMMONS and submitted by the due date. Failure to provide the updated by the due date could jeopardize this funding offer.

Sincerely,

Gregory P. Smith
Lead Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2119, MSC 7614
Bethesda, Maryland 20892-7614
Overnight Mail Only: Use Zip 20817
Phone: (301) 402-5540
Fax: (301) 493-0597
Email:gs225y@nih.gov

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Obtained via FOIA by White Coat Waste Project

From: Smith, Gregory (NIH/NIAID) [E]
To: "plowe@k-state.edu"
Cc: rganta@ksu.edu; Smith, Gregory (NIH/NIAID) [E]
Subject: FW: URGENT JIT Update Requested RE: 2R01AI070908-06A1
Date: Tuesday, July 22, 2014 4:38:36 PM
Attachments: Automatic reply URGENT JIT Update Requested RE 2R01AI070908-06A1.msg
Importance: High

Hi Paul,

I received an out of office for the PI (attached). Please assist in getting the expired COMMONS JIT revised and submitted by the due date.

Thanks!

Greg Smith
Lead Grants Management Specialist
DHHS/NIH/NIAID/GMP
Phone: (301) 402-5540
Fax: (301) 493-0597
gs225y@nih.gov

From: Smith, Gregory (NIH/NIAID) [E]
Sent: Tuesday, July 22, 2014 4:32 PM
To: 'rganta@ksu.edu'; 'plowe@k-state.edu'
Cc: Perdue, Samuel (NIH/NIAID) [E]; Smith, Gregory (NIH/NIAID) [E]
Subject: URGENT JIT Update Requested RE: 2R01AI070908-06A1

Dear Dr. Ganta and KSU Business Officials:

Congratulations! Your above referenced grant was selected for 4 years of discretionary funding. **The following JIT request does not guarantee your grant will be funded. Any official notification of funding can only be provided by a Notice of Grant Award. Be advised we are operating in our end of fiscal year processing so funding decisions must be made quickly.**

Your JIT originally submitted for this grant is greater than 120 days old and must be revised/update to be considered valid. The revised JIT should be submitted no later than end of business on July 25, 2014:

Please ensure your updated JIT includes the following:

- Complete Other Support for all active and pending projects for Dr. Roman R. Ganta
- Current IACUC approval date

As requested the JIT should be updated in the COMMONS and submitted by the due date. Failure to provide the updated by the due date could jeopardize this funding offer.

Sincerely,

Obtained via FOIA by White Coat Waste Project

From: Roman Ganta
Sent: Tue, 22 Jul 2014 20:32:06 +0000
To: Smith, Gregory (NIH/NIAID) [E]
Subject: Automatic reply: URGENT JIT Update Requested RE: 2R01AI070908-06A1

Please note that I will be away from my office from July 12 to August 5 and my e-mail access will be limited. If anything requires immediate attention, please contact our department office. Thank you.

Roman Ganta

Obtained via FOIA by White Coat Waste Project

Gregory P. Smith
Lead Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2119, MSC 7614
Bethesda, Maryland 20892-7614
Overnight Mail Only: Use Zip 20817
Phone: (301) 402-5540
Fax: (301) 493-0597
Email:gs225y@nih.gov
Disclaimer:
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Obtained via FOIA by White Coat Waste Project