

SUMMARY STATEMENT
(Privileged Communication)

PROGRAM CONTACT:

Release Date: 11/28/2016

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Application Number: 1 R15 AI126385-01A1

Principal Investigator

RAGHAVAN, RAHUL

Applicant Organization: PORTLAND STATE UNIVERSITY

Review Group: ZRG1 IDM-S (81)
Center for Scientific Review Special Emphasis Panel
AREA applications in Infectious Diseases and Microbiology

Meeting Date: 11/07/2016
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Requested Start: 04/01/2017

RFA/PA: PA16-200
PCC: M46C B

Project Title: Elucidating the evolution of Coxiella to uncover critical metabolic pathways

SRG Action: Impact Score: [REDACTED]
Next Steps: Visit http://grants.nih.gov/grants/next_steps.htm
Human Subjects: 10-No human subjects involved
Animal Subjects: 10-No live vertebrate animals involved for competing appl.

Project Year	Direct Costs Requested	Estimated Total Cost
1	[REDACTED]	[REDACTED]
TOTAL	[REDACTED]	[REDACTED]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

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1R15AI126385-01A1 Raghavan, Rahul

RESUME AND SUMMARY OF DISCUSSION: This resubmitted application proposes to identify critical metabolic pathways for intracellular growth of *Coxiella burnetii* using a comparative genomic approaches. The results could increase the basic understanding of survival mechanism of *C. burnetii* in the phagosomal vesicles in host cells. The application can also have strong implications on the study of pathogenesis of other tick-borne pathogens. The investigator has adequately addressed the previous concerns. This submission has a clear prioritization plan. The strong preliminary data, though not required, provide the application with a strong premise. The strengths are that the investigator is very productive and has a solid publication record; the hypothesis that critical metabolic pathways may be acquired through horizontal gene transfer is innovative; the comparative genomic analysis between *C. burnetii* and other nonvirulent tick-associated *Coxiella* is well described with adequate rigor; a transposon-generated mutant library is already available to screen functionally active genes critical for intraphagosomal growth of *C. burnetii*; the potential for student involvement in research is excellent; and the environment is appropriate. The overall enthusiasm is very high for this application proposing to identify critical metabolic pathways for intracellular survival of *C. burnetii*.

DESCRIPTION (provided by applicant): *Coxiella burnetii*'s pathogenicity depends on its ability to grow in a lysosome-derived hostile vacuole within human cells. However, metabolic processes critical to *C. burnetii*'s intracellular growth are largely unknown. This lack of knowledge has prevented both the understanding of its basic biology and pathogenesis, and the development of better therapeutic agents. The long-term goal is to understand the molecular details of *Coxiella*'s distinctive physiology, and to apply this knowledge to developing novel therapeutic strategies. The objective of this application is to identify metabolic pathways that are vital to *C. burnetii*'s intracellular growth. The central hypothesis, which was formulated based on preliminary data, is that *C. burnetii* evolved from a tick-associated ancestor by acquiring critical metabolic genes through horizontal gene transfer (HGT). A novel evolutionary genomics approach will be used to identify metabolic pathways that are critical to *C. burnetii*'s intra- cellular growth. The rationale for the proposed research is that once metabolic processes important to *C. burnetii*'s intracellular growth are identified, pharmacological agents that block these pathways could be developed to treat chronic infections more effectively. The objective of this project will be accomplished by three specific aims: (1) Identify metabolic pathways that distinguish *C. burnetii* from tick-associated *Coxiella*. The working hypothesis is that genes critical to *C. burnetii*'s intracellular physiology will not be present in avirulent tick-associated *Coxiella*. The genome of a closely related *Coxiella* from the tick *Ornithodoros rostratus* will be sequenced and compared to *C. burnetii*'s genome. (2) Define metabolic pathways that are critical to *C. burnetii*'s intracellular growth. The working hypothesis is that genes acquired via HGT are being maintained in *C. burnetii* because they are critical to the pathogen's physiology. Phylogenetic approaches will be used to identify HGT-derived genes, and their functions will be validated using RNA-seq and genetic tools. (3) As a proof of principle, determine the importance of heme biosynthesis to *C. burnetii*'s intracellular growth. The working hypothesis is that heme biosynthesis is crucial to *Coxiella*'s growth. Heme production and intracellular growth of heme pathway-deficient strains will be assayed. This study is innovative because it (a) uses a novel approach that overcomes the current limitations in studying *Coxiella* at a genome-wide scale, and (b) is based on a novel concept that the human pathogen evolved from a tick symbiont via massive HGT. The proposed project is significant because it will (a) uncover metabolic pathways that are critical to the pathogen's intracellular growth, (b) identify new therapeutic targets, for example, HemA and HemL are essential for heme biosynthesis in *C. burnetii* but are not present in humans cells, (c) provide a model approach for identifying genes involved in host adaptation, which could be applied broadly to other pathogens such as *Francisella* and *Rickettsia* spp. where avirulent tick symbionts could be compared to virulent human-specialized strains.

PUBLIC HEALTH RELEVANCE: *Coxiella burnetii* is a zoonotic bacterium that causes Q fever and chronic endocarditis. Metabolic processes critical to its intracellular growth and pathogenesis are not

known. The proposed project will identify important metabolic pathways in *C. burnetii* using a novel evolutionary genomics approach that will overcome the current technical difficulties in studying *Coxiella* at a genome-wide scale. The application will also provide a useful model for identifying key metabolic processes in other human pathogens and will identify new genes that could be targeted to treat chronic Q fever more effectively.

CRITIQUE 1:

Significance: 2
Investigator(s): 1
Innovation: 1
Approach: 1
Environment: 2

Overall Impact: This innovative revised application aims to identify the metabolic pathways critical for *Coxiella burnetii* for its intracellular/phagosomal growth. The investigator's hypothesis is that the pathogen acquired critical metabolic pathway genes by horizontal gene transfer (HGT). Previous studies by the investigator provide a solid premise for this exciting hypothesis. The project has three well-focused and independent specific aims. Novel data are expected at the end of this study. Previous submission was also considered as highly innovative and that the investigator was identified as well-qualified to carry out the proposed project goals. The investigator responded very well in addressing the prior noted minor concerns. Enthusiasm for this revised application remained strong.

1. Significance:

Strengths

- *Coxiella burnetii* is an important human pathogen, as it is capable of causing significant morbidity and mortality.
- As very few organisms are capable of causing the disease, Q fever, and that the organism survives well in the environment for very long periods of time, this pathogen is listed as a BSL3 select agent.
- The proposed project outcomes may also be valuable in initiating similar studies in understanding the molecular basis for pathogenesis of several other tick-borne pathogens identified in recent years as causing serious diseases in people.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- The investigator is an accomplished young scientist who has the necessary expertise.
- His record of productivity up to this point is also excellent and it is directly relevant for studying the bacterial genomes and other proposed experiments.

Weaknesses

- None noted.

3. Innovation:

Strengths

- The project is novel as it aims to identify metabolic pathways critical for *Coxiella* for its intracellular/phagosomal growth.
- Non-pathogenic endosymbionts also co-exist in ticks along with pathogenic organisms belonging to the genera *Rickettsia*, *Ehrlichia* and *Anaplasma*. Comparative genomics proposed in the current study on two *Coxiella* species may open ways for similar research focused on understanding how other pathogenic bacteria are evolved in ticks by co-existing with non-pathogenic microbiomes.

Weaknesses

- None noted.

4. Approach:

Strengths

- This study aims to identify metabolic pathways vital for *C. burnetii*'s intracellular growth. Comparative genomics will be performed by analyzing a soft tick-derived endosymbiont species of *Coxiella* and *C. burnetii* genomes to identify plausible metabolic pathway genes acquired for pathogenesis by horizontal gene transfer (HGT) (aim 1).
- In aim 2, RNA sequence data from wild type and transposon mutant libraries will be generated and evaluated to map functionally active genes critical for the intraphagosomal growth. This goal is independent of the first aim and a transposon library is already available to screen.
- Prior studies by the investigator's team already identified heme biosynthesis pathway as having HGT genes, which are absent in the *Coxiella* endosymbionts in ticks. The investigator will directly test the hypothesis by evaluating this pathway for its role in intracellular growth (aim 3).
- The study is likely to open new research directions in defining how the pathogenic organisms are evolved in ticks and in other arthropod vectors.
- The proposed project goals in defining the metabolic pathways acquired by HGT in pathogenic bacteria are novel.
- The study is likely lead to a better understanding of how other disease causing agents are evolved.
- Alternate methods are well described.

Weaknesses

- None noted.

5. Environment:

Strengths

- Excellent

Weaknesses

- No concern.

Protections for Human Subjects:

Not Applicable

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

No concern

Resubmission:

- This is a revised application and the investigator addressed all of the prior concerns.

Select Agents:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Significance: 3

Investigator(s): 1

Innovation: 2

Approach: 2

Environment: 1

Overall Impact: This is a well-written application that aims to use evolutionary genomics approach to identify metabolic pathways that are critical to the intracellular growth of *Coxiella burnetii*. The investigator plans to test his hypothesis, which *C. burnetii* evolved from a tick-associated ancestor by acquiring metabolic genes through horizontal gene transfer, by the following three specific aims: 1) Identify metabolic pathways that distinguish *C. burnetii* from the tick-associated *Coxiella*. 2) Define metabolic pathways that are critical to *C. burnetii*'s intracellular growth. 3) Determine the importance of heme biosynthesis to *C. burnetii*'s intracellular growth. The study, if successful, may give insights on metabolic pathways for intracellular growth and new therapeutic targets for *C. burnetii*. This application is likely to produce useful data since the investigator has already obtained some preliminary data and collaborated with eight collaborators. The investigator has a track record of manuscript publications and training undergraduate and graduate students.

1. Significance:

Strengths

- Identification of metabolic genes that are acquired by horizontal gene transfer is important for the intracellular growth of *C. burnetii*. The genome-scale approach of identifying metabolic genes using genome sequences from virulent *C. burnetii* and tick-associated *Coxiella* symbiont could be applied to other tick pathogens, such as *Rickettsia* and Francisella species.
- The tRNA-acquisition-based virulence mechanism studied in this study could serve as a model of studying other pathogenic bacteria, including *Corynebacterium* and *Clostridium* species.

Weaknesses

- The genome-scale approach of identifying different genes between virulent and symbiotic bacteria have been explored in other organisms in the past.

2. Investigator(s):

Strengths

- The investigator has expertise in bioinformatics, high-throughput sequencing, and microbiology. All of them are important to the success of the proposed studies.
- The investigator has a track record of manuscript publications. Since the investigator became an assistant professor at PSU in 2012, he has published four manuscripts on *Coxiella*.
- There are six support letters from collaborators in this application. All of the collaborators have the expertise in the proposed experiments. These are important for the successful completion of this research proposal.

Weaknesses

- None noted.

3. Innovation:

Strengths

- Metabolic pathways that are critical for intracellular growth of *C. burnetii* have not been explored.
- The concept that virulence factors and metabolic genes of *C. burnetii* were obtained by horizontal gene transfer is novel.
- The use of a novel tRNA-acquisition-based virulence mechanism has not been explored in *C. burnetii*.

Weaknesses

- Identification of metabolic pathways for intracellular growth by genome comparisons has been explored in other organisms.
- Figure 3: Although *C. burnetii* could evolve from a tick-associated ancestor by acquiring genes through horizontal gene transfer, *C. burnetii* could obtain those genes from an environmental ancestor by horizontal gene transfer and later became tick-associated symbionts by gene loss.

4. Approach:

Strengths

- The design and methods of this application is appropriate.

- Preliminary data are available for all three specific aims. The preliminary data support the investigator's hypothesis to be tested and feasibility of the proposed research.
- Potential pitfalls and alternative methods are discussed for all three specific aims.

Weaknesses

- The investigator proposed to sequence the genome of a *Coxiella* species in *Ornithodoros rostratus*. Although the genome sequences will help the investigator to identify metabolic genes that are only present in *C. burnetii*, the specific aim could be carried out with the sequenced genomes of *Coxiella* in *Amblyomma americanum* and *Coxiella* in *Rhipicephalus turanicus*.
- The history of *O. rostratus* lab colony is not known. The genetic elements might be different between wild type and lab colony of *O. rostratus*.

5. Environment:

Strengths

- The investigator's lab is located in a new research facility that is shared between Portland State University, Oregon Health and Science University, and Oregon State University. The unique location maximizes the probability of resource utilization and promotes collaborations among faculty members from three universities.
- Two Linux servers are available for genome sequencing project and annotation.
- The investigator has access to all core facilities in Oregon Health and Science University.
- The investigator received a reduced teaching load prior to his evaluation for tenure.

Weaknesses

- None noted

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Resubmission:

- Appropriate addressed

Select Agents:

Acceptable

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

Significance: 2

Investigator(s): 2

Innovation: 2

Approach: 2

Environment: 2

Overall Impact: The resubmission outlines experiments to understand the evolution and pathogenesis of *Coxiella burnetii*. This intracellular pathogen causes acute flu-like symptoms, and can cause endocarditis. Illness is difficult to diagnosis and treat, with antibiotic resistances becoming more common. This is a select agent, with bio-terrorism threat. No vaccine is available, and thus significance is considerable, though disease is limited in the US. *C. burnetii* can survive the lysosomal compartment as a small cell variant. The closest relative to *C. burnetii* is found within ticks, and it is proposed that this relative evolved to be able to infect humans. The investigator has a solid publication record in the areas necessary for carrying out the research. The approach is innovative, made possible by the capabilities of nextgen sequencing, RNAseq, and analysis software. The investigator proposes to investigate metabolic changes that have allowed for *Coxiella* virulence in humans to appear. The investigator will do whole genome sequencing of the tick-associated *Coxiella* and two genomes from *C. burnetii* isolates and then compare this to existing *C. burnetii* genomes, with the hypothesis that metabolic differences can, in part, explain human host adaptation. The group will use KEGG and other software analysis, and focus on heme metabolism. The have identified a tRNAGlu, acquired by horizontal gene transfer, thought to be involved in fitness. The investigator, with a solid track record of publishing, is highly experienced in genomic analysis, and the use of software such as KEGG for understanding the differences between the *Coxiella* species is a solid approach. To gain a greater understanding of PV survival and the metabolic pathways necessary for pathogenesis, the investigator proposes using bioinformatics and then RNAseq to confirm their observations concerning gene expression within human macrophage. The experiments proposes are comprehensive, addressing questions from multiple angles, and are thus scientifically rigorous. The application would be impactful in training undergraduate and graduate students.

1. Significance:

Strengths

- *C. burnetii* is the causative agent of Q fever
- Extremely low infectious dose, with increased reported infections in the US
- Select agent, a bioterrorism threat, no vaccine available

Weaknesses

- Somewhat limited disease in the US

2. Investigator(s):

Strengths

- Productive, early stage investigator
- Already has an established track record of mentoring students
- Highly experienced in the assembly and analysis of genomic sequences

Weaknesses

- None noted

3. Innovation:

Strengths

- Studying the evolutionary history of *C. burnetii* from a tick-associated relative
- Investigating metabolism as it relates to pathogenesis, and how *C. burnetii* survives within the phagolysosome
- Chemical biology approaches for targeting heme production in future experimentation offer sound approach for intervention

Weaknesses

- Limited, though increasing disease incidence in the US.

4. Approach:

Strengths

- Comparative genomic analysis to determine the genes required for *C. burnetii* pathogenesis in humans in comparison to the tick-associated *O. rostratus* most likely will be informative
- Collaboration in place for acquiring *O. rostratus* material and genetic manipulations
- Identification of target metabolic pathways through bioinformatics, then testing expression within the phagolysosome adds scientific rigor.

Weaknesses

- Construction of genetic mutants using Tn mutagenesis could prove challenging because of lethality, etc., though alternate approaches are in place for construction and then testing in the macrophage cell line
- Not clear how horizontally acquired genes are being identified, except that they are not from Gamma proteobacteria.

5. Environment:

Strengths

- All the resources are in place to carry out the research, with collaborative interaction with investigators within and outside the institution
- The application would greatly enhance the research capability of the institution
- Excellent opportunities for undergraduate and graduate student training

- Partial teaching reduction would allow for focused research effort by the PI

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

- BSL-2 facilities and autoclave are in place for the research.

Resubmission:

- The investigator has addressed a number of concerns, both with the significance as well as with the approach. The significance is reiterated by the investigator, with implications to other intracellular pathogens. For the approach, a number of issues have been addressed, concerning how to interpret the data and directions to proceed. The potential impact to the institution, in this reviewer's opinion, will be significant.

Select Agents:

Acceptable

- The investigator states that all work will use the *Coxiella burnetii* strain to be used for the investigation is avirulent and exempt (Nine Mile Phase II strain (RSA 439). Propagation of this strain will occur under BSL2 containment.

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 R15 AI126385-01A1; PI Name: Raghavan, Rahul

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html>. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.