FOR OFFICE USE ONLY: IACUC Protocol # [redacted] Application Received: Routed: 8/13/21 Approved: 9/18/20 Expires: 9/17/23

USDA Covered X Exempt IBC # [redacted]

KANSAS STATE UNIVERSITY
University Research Compliance Office

Institutional Animal Care and Use Committee (IACUC)
Application for Approval Form

Please send your completed application to comply@k-state.edu

Version: Last Updated: 02/24/2020

ADMINISTRATIVE INFORMATION:

Responsible Individual/PI: Roman R. Ganta

Responsible Graduate Student (if applicable):

Title of Project/Course: Vector and host contributions to *Ehrlichia chaffeensis* gene expression

Species/ Strain to be used: dog

Type of Application: ☑ New ☑ Renewal of IACUC# [redacted] For a renewal, check both "New" & "Renewal"

☐ Addendum/Modification (complete modification section below)

Category: (check one box) ☐ Teaching ☐ Testing ☑ Research

☐ Other (if other, describe)

Funding Source: ☑ PHS/NIH ☐ Other Federal Agency List:

☐ State ☐ Other List:

Principal Investigator: Roman R. Gantra Degree/Title: M.S., Ph.D./Professor

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Co-Principal Investigators:

Name: Dept: Degree/Title:

Name: Dept: Degree/Title:

MODIFICATION:

If you are requesting a modification or a change to an IACUC approved protocol, please provide a concise description of all of the changes that you are proposing in the following block. Additionally, please highlight or bold the proposed changes in the body of the protocol where appropriate, so that it is clearly discernible to the IACUC reviewers what and where the proposed changes are. This will greatly help the committee and facilitate the review.

I submit the following minor modification request (August 12, 2021)

Please add the following persons to the protocol: Swetha Madesh, Cheyenne Knox, Shawna Fitzwater

Please delete Arathy Nair. Thank you.

We submit the following minor modification request (May 11, 2021):

Please add Ms. Ashley Thackrah. Please delete Dr. Anamika Guptha. Thank you.

I. NON-TECHNICAL SYNOPSIS (Please provide a brief narrative description of proposal. This should typically be less than 75 words and be easily understood by nonscientists, e.g. `*We propose to test the effectiveness of a new class of anti-inflammatory drugs against arthritis that develops in the hips of dogs affected by congenital hip dysplasia*'):

Goals of this protocol are to evaluate tick transmitted Ehrlichia chaffeensis (a Gram negative bacterium) mutants to discover proteins involved in causing the disease; human and canine monocytic ehrlichiosis, using the dog infection model. Dog studies are preferred as the pathogen causes disease in this host similar to people. The study outcomes will be important in developing disease preventing strategies in people and dogs.

II. BACKGROUND (concise narrative review of the literature and basis for the study):

Dog is chosen as the infection model for the proposed experiments because it is an incidental host in acquiring *Ehrlichia chaffeensis* similar to humans. Moreover, our recent experimental studies demonstrated that this host serves as an excellent infection model, where the pathogen infection persists in the host and without causing a severe disease. In particular, our recent experimental infection studies demonstrated that dogs develop only mild fever (rise in only up to 1.5^{o}C body temperature), while maintaining persistent infections with detectable hematological changes, host response and pathology.

This application is to replace our soon to be expired IACUC application # [redacted]. This renewal application will allow us to complete the experiments listed as part of protocol #[redacted]. The goals of application I#[redacted] are to perform *in vivo* screening studies to identify genes essential for the *E. chaffeensis* pathogenesis. The current proposed application will use the same protocols as outlined in the previous application, which is a part of the funded NIH R01 grant # **Grant Number: 2R01AI070908-10** and the grant will expire on **06/30/2024**. ***We used protocol#*** [redacted] ***to assess the vaccine potential of insertion mutations in E. chaffeensis that cause attenuated growth in dogs against wild-type infection challenge*** [1]***. We also defined the role of the immune system in vaccine-induced protection from E. chaffeensis infection in dogs; 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 in dogs*** [2]**. We used protocol #**[redacted] **to identify significant expansion of a pathogen-specific double positive T-cell population in dogs that are persistently infected with *E. chaffeensis*** [3]. **Further, studies carried out as part of this protocol also aided in defining how mutations in *Ehrlichia chaffeensis* in causing polar effects in gene expression and differential host specificities [4]. In addition, our studies helped to determine *Amblyomma americanum* ticks infected with *in vitro* cultured wild-type and mutants of *Ehrlichia chaffeensis* serve as competent vectors to produce infection in naïve dogs [5].** The currently active protocol #[redacted] for which we are requesting renewal **was used to perform studies to identify many genes critical for *E. chaffeensis* persistent *in vitro* growth [6]. (This publication is also identified by the journal editors as a 'Spotlight' article of the issue.)** This new IACUC application goals are the same in application #[redacted]; we request approval to complete the remaining unfinished portion of the project. **Total number of dogs requested and approved for accomplishing the goals of all three experiments was 126 (60+36+30) as per the protocol #**[redacted]**. As indicated above, we completed assessing a subset of mutants using 18 dogs as part of experiment 1 of this protocol. With this, we propose to reduce the total number of dogs for experiment 1 to 42, thus the total number of animals proposed is now 108.**

The family *Anaplasmataceae* contains several obligate, intracellular, Gram-negative bacteria which include species of the genera *Ehrlichia* and *Anaplasma* [5]. A steady increase of potentially

fatal human diseases caused by t*Ehrlichia* and *Anaplasma species* infecting phagocytic cells has been reported during the last 25 years [6][7]. They include three tick-transmitted emerging diseases of humans caused by *Ehrlichia* species: human monocytic ehrlichiosis (HME) caused by *E. chaffeensis* [[8],[9], human ewingii ehrlichiosis caused by *E. ewingii* [10], and a more recently identified infections with *E. muris subspecies* eauclairensis [11]. Further, infections in people are also caused by *Anaplasma phagocytophilum* resulting in the disease, human granulocytic anaplasmosis (HGA)[12][13]. *E. chaffeensis, E. ewingii, E. canis,* and *A. phagocytophilum* also cause diseases in dogs. Tick-transmitted pathogens have evolved strategies to persist in both tick and vertebrate hosts in order to successfully complete their infectious cycle.

We and other researchers presented evidence that *Ehrlichia* species alter protein expression for many proteins in a host cell- specific manner [14] ,[15] [16]. 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 [17]. Furthermore, the host response differed considerably from *E. chaffeensis* originating from tick and vertebrate host environments [17]. 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 infection acquisition, and understanding regulation of the pathogen gene expression in vertebrate and invertebrate host environments.

We recently performed mutational analysis and demonstrated that mutations in three different genes of *E. chaffeensis* caused attenuated growth of the organism *in vivo* [18]. These data formed the basis for our funded NIH-R01 grant application having the following three 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. Specific aims 2 and 3 involve the use of experimental infection studies in dogs. We already completed the goals of aim 2 as part of our previously expired IACUC application # [redacted]. Further, we have completed some of the proposed experiments of aim 3 as part of IACUC application # [redacted]. This application will focus on the remaining proposed experiments of aim 3.

1. 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. Infect Immun. 2015;83.

2. McGill JL, Nair ADS, 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. 2016;11.

3. 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. Front Immunol. 2018;9. doi:10.3389/fimmu.2018.01585.

4. Cheng C, Nair ADS, Jaworski DC, and Ganta RR. Mutations in *Ehrlichia chaffeensis* causing polar effects in gene expression and differential host specificities. PLoS ONE (2015 Jul 17;10(7):e0132657. doi: 10.1371/journal.pone.0132657. eCollection 2015. PMID: 26186429).

5. 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: 27729288

6. Wang Y, Nair ADS, Alhassan A, Jaworski DC, Liu H, Trinkl K, et al. Multiple Ehrlichia chaffeensis genes critical for its persistent infection in a vertebrate host are identified by random mutagenesis coupled with in vivo infection assessment. Infect Immun. 2020. doi:10.1128/IAI.00316-20.

5. Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, et al. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of

Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combi. Int J Syst Evol Microbiol. 2001;51:2145 -65. doi:10.1099/00207713-51-6-2145.
6. Dumler JS, Bakken JS. Ehrlichial Diseases of Humans: Emerging Tick-Borne Infections. Clin Infect Dis. 1995;20:1102 -10. doi:10.1093/clinids/20.5.1102.
7. McDade JE. Ehrlichiosis--A Disease of Animals and Humans. J Infect Dis. 1990;161:609 -17. doi:10.1093/infdis/161.4.609.
8. Dawson JE, Anderson BE, Fishbein DB, Sanchez JL, Goldsmith CS, Wilson KH, et al. Isolation and characterization of an Ehrlichia sp. from a patient diagnosed with human ehrlichiosis. J Clin Microbiol. 1991;29:2741 -5. doi:10.1128/JCM.29.12.2741-2745.1991.
9. Maeda K, Markowitz N, Hawley RC, Ristic M, Cox D, McDade JE. Human Infection with Ehrlichia canis , a Leukocytic Rickettsia. N Engl J Med. 1987;316:853 -6. doi:10.1056/NEJM198704023161406.
10. Buller RS, Arens M, Hmiel SP, Paddock CD, Sumner JW, Rikihisa Y, et al. Ehrlichia ewingii, a Newly Recognized Agent of Human Ehrlichiosis. N Engl J Med. 1999;341:148 -55. doi:10.1056/NEJM199907153410303.
11. Pritt BS, Sloan LM, Johnson DKH, Munderloh UG, Paskewitz SM, McElroy KM, et al. Emergence of a New Pathogenic Ehrlichia Species, Wisconsin and Minnesota, 2009. N Engl J Med. 2011;365:422 -9. doi:10.1056/NEJMoa1010493.
12. Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic Ehrlichia species as the etiologic agent of human disease. J Clin Microbiol. 1994;32:589 -95. doi:10.1128/JCM.32.3.589-595.1994.
13. Choi K, Grab DJ, Dumler JS. Anaplasma phagocytophilum Infection Induces Protracted Neutrophil Degranulation. Infect Immun. 2004;72:3680 -3. doi:10.1128/IAI.72.6.3680-3683.2004.
14. Ganta RR. Molecular characterization of Ehrlichia interactions with tick cells and macrophages. Front Biosci. 2009;Volume:3259. doi:10.2741/3449.
15. Seo G-M, 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. Infect Immun. 2008;76:4823 -32. doi:10.1128/IAI.00484-08.
16. Singu V, Liu H, Cheng C, Ganta RR. Ehrlichia chaffeensis Expresses Macrophage- and Tick Cell-Specific 28-Kilodalton Outer Membrane Proteins. Infect Immun. 2005;73:79 -87. doi:10.1128/IAI.73.1.79-87.2005.
17. Ganta RR, Cheng C, Miller EC, McGuire BL, Peddireddi L, Sirigireddy KR, et al. Differential Clearance and Immune Responses to Tick Cell-Derived versus Macrophage Culture-Derived Ehrlichia chaffeensis in Mice. Infect Immun. 2007;75:135 -45. doi:10.1128/IAI.01127-06.
18. Cheng C, Nair ADS, Indukuri V V., Gong S, Felsheim RF, Jaworski D, et al. Targeted and Random Mutagenesis of Ehrlichia chaffeensis for the Identification of Genes Required for In vivo Infection. PLoS Pathog. 2013;9:e1003171. doi:10.1371/journal.ppat.1003171.

III. LITERATURE SEARCH FOR UNNECESSARY DUPLICATION

(If your proposed activity is part of the formal veterinary teaching curriculum and is not research or testing, you may not have to perform a literature search for unnecessary duplication. A non-duplication explanation for teaching projects, for III.D., can be found at: https://www.k-state.edu/comply/iacuc/resources/protocol-development/teaching-projects/index.html.
A literature search for unnecessary duplication is required for all proposed research activities using animals.)

A. Date of literature search (should be within the last month): 08/19/2020

B. Search at least two appropriate databases and provide the years of coverage (i.e., PubMed (1950/current), CAB (1910/present)). A list of databases is available online at http://guides.lib.k-state.edu/sb.php?subject_id=38563:

1) Pubmed (1950-present)

2) CAB Direct (1920-present)

3)

C. Keywords/Search Strategy:

Dog, *Ehrlichia chaffeensis*, Tick transmission, gene expression

D. Please provide a concise narrative of the results of the searches relative to unnecessary duplication. You do not need to provide a copy of the actual search with your proposal, but it should be maintained for your records or available to the IACUC if requested. Gayle Willard, Dir, Vet Med Library is the IACUC consultant. Please contact her if you need assistance. Phone 2-6006; email: gwillard@vet.ksu.edu

Concise Narrative:

The search with the above search terms resulted in two publications in Pubmed and 1 in CAB Direct. None of them revealed any possible alternatives to infection studies

IV. OBJECTIVE\HYPOTHESIS (briefly state the objective of the study - and, if applicable, the hypothesis to be accepted or rejected):

The objectives of this IACUC application are to perform in vivo screening of *E.chaffeensis* mutants to identify genes essential for pathogens survival in vertebrate and tick hosts.

V. MATERIALS AND METHODS:

V.A. Experimental Design and General Procedures (succinctly outline formal scientific plan for study)**:**

Animal Details: Species, strain, sex, age, weight, number
Study Timeline: Arrival of animals, acclimation period, start of study, study time points, end of study
Biosample Details: Type, amount, method, location, needle size

Objective: Perform mutational analysis and *in vivo* screening to identify genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts. We will generate large pools of *E. chaffeensis* transposon mutants in support of this objective. Our funding was approved to generate 200 mutant organisms. These mutants will then be screened to define the pathogenesis using the canine infection model; three experiments were proposed to accomplish this goal.

Experiment 1) We have screened 60 mutants in 6 pools by infecting three dogs with about 10 mutants in each pool. A total of 18 dogs has been used under this objective as part of the current protocol #[redacted]. In this protocol, we propose to infect up to 14 pools of mutants (each pool of up to 10 mutants) each in three independent animals (n=3) using a total of 42 animals. The infection status will be assessed twice a week for two months. Nymphal ticks (typically about 250) will be allowed to acquisition feed on animals starting from day 5 post infection. Tick cells (containers that hold ticks) will be placed on dogs and covered with sheep soc (made of Nylon Spandex for easy flexibility) (Sheepman Supply co. or something similar) by following the procedures similar to those done on deer, except that there is no need for anesthetize the dogs. For these experiments, the backs of the animals will be shaved with veterinary clippers. A custom designed tick containment chamber (modified top of Nalgene jar containing screw cap lid) will be glued to polyvinyl membrane with a center circular opening. The chamber will then be glued to animals with industrial adhesive (commercially available). The chambers have round bottom

smooth surface and once glued, the chambers remain attached for several weeks until polyvinyl membrane is lifted off the skin with the hair growth. To ensure that the chambers are tightly attached, tick infestations will be performed only after about 24 h following the attachment of the chambers. In particular, we will monitor for the retainment of the chambers on the animals, as well as their firm attachment. If dogs attempt to remove the chambers, we will place Elizabethan neck collars to restrict grooming. The chambers will be covered with sheep sox. To perform the tick infestation, lids of the chambers will be unscrewed, ticks will be placed inside and the chambers will then be tightly closed with the lids and animals will be covered back with sheep sox. 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 (10 ml blood drawn twice a week from cephalic veins 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 (total dogs for this sub-experiment are 42).

Experiment 2) We will repeat the infection experiment on all those mutants identified as attenuated *in vivo* in the previous experiment. As in the previous experiment, 10 ml blood samples will be collected from cephalic veins and tick attachment experiment will also be performed and essentially as outlined above. We will clonally purify the mutants, randomly select pools containing 5 clones each and use them to repeat the infections in three dogs (n=3) each as in the previous experiment. We expect to identify about 60 mutants from the first experiment and pools of 5 mutants each will be used per dog; thus, the total pools for this second experiment will be 12. Twelve experiments with three dogs per experiments and the total number of dogs will be 36.

Experiment 3) 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 for this experiment). Similar to experiments 1 and 2, blood draws (10 ml each time from cephalic veins) and tick attachment experiments will be performed as outlined above 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 of all three experiments will be 108 (42+36+30).

In all three experiments, the animals will be kept for 60 days each to monitor the mutant *E. chaffeensis* circulation in blood. Blood sampling will be done twice a week from cephalic veins (10 ml each) for the first two weeks and then once a week thereafter. Total blood draws will be 11 times per animal.

About 6 to 8 month-old dogs of the breed 'Beagle' will be used for these experiments. For convenience, we will either use all males or all females in each experimental group, while maintaining equal numbers of males and females throughout the study. The weight of each animal will be about 15 to 20 pounds. Diphenhydramine (Benadryl) (1mg per pound) will be orally administered to all animals about 30 minutes prior to inoculation with Ehrlichia. (The stock concentration to be used is 2.5 mg/ml; 6 to 8 ml per animal for 15 to 20 pound dogs.) Benadryl is administered to prevent any possible anaphylactic shock resulting from injection of organisms containing traces of serum or other animal products likely present in the culture media.

The terminal bleed (by heart puncture method) serum is the only source to collect up to 50 ml of blood-derived high titer sera from animals. We request this option to draw blood just prior to euthanasia. When possible, we will do that via cephalic or saphenous vein. We will need to sedate the dogs with Dexmedetomidine hydrochloride (Dexdormitor) 0.5 mg/ml at a dose of 23-25 mcg/kg (0.4-0.5 ml/dog) for IM injection or 16.8-19.6 mcg/kg (0.3-0.4 ml/dog) for IV injection and draw the blood via cardiac puncture once dog is complete sedated.

V.B. Photos, Videos and/or Audio Recordings (IACUC Guideline #6 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/6.html)**:**

1. Will you be taking any photos, videos and/or audio recordings? ☐ Yes ☑ No

2. Please provide the necessary details to explain what you will be taking photos, videos and/or audio recordings of.

3. Please explain how the photos, videos and/or audio recordings will be used.

4. Please describe how the photos, videos and/or audio recordings will be stored.

V.C. Non-animal Alternatives Considered (were non-animal alternatives considered - why are they not used?):

It is not possible to have any non-animal alternatives for understanding the disease progress comparisons, immune responses and pathogens' persistence.

V.D. Animal Model and Species/Strain Justification (Explain why animals are needed for your study. Give your rationale and justification for selecting this animal model or species):

Dogs is the perfect animal model for such studies because it acquires *E. chaffeensis, E. canis and A. phagocytophilum* infections naturally like humans and moreover tick transmission studies can be done in this animal model similar to those likely occurring in people. Furthermore, the dog model supports the development of persistent infections with all three pathogens. The Beagle breed is chosen for this study because it is the most commonly reported breed for similar studies in the literature and moreover, it is easy to work with this breed. Finally, this dog breed is commercially available for use in experimental studies.

V.E. Animals Requested -used in research testing or teaching (list genus and species/strain of animal model proposed)**:**

Genus and Species:	*Canis familiaris*
Total number (by species) requested: (this should correspond to the sum of the animals listed in Section VI.A. below. The IACUC approves protocols for a period of 3 years, so the number(s) listed here should represent the TOTAL number of animals requested for a project up to a three-year period- and not simply reflect annual usage projections.)	108
Source of animals (by species):	We will use a commercial class USDA licensed dealer to obtain dogs.

V.F. Justification of Animal Numbers / Data: Analysis: Research, testing, and teaching activities should be designed to provide a statistically significant result with a minimum number of animals. The specific method by which the number of animals was determined must be clearly stated. Statistical techniques and/or power analysis are appropriate in most cases to maximize the usefulness of the data generated from each animal. However, the IACUC acknowledges that the basis for an appropriate justification of animal numbers depends largely on the nature of the study itself. Prior experience and expertise with the model in question may be taken into account as well, but must be carefully documented in the protocol. The cost of the animal should not be considered as the primary justification for the use of a particular species or model. Consultation with a biostatistician or use of statistical software during the design phase of the experiment may be useful. This website may be helpful in performing a power analysis: http://statpages.org

Five basic types of studies are listed below, along with brief general guidelines for the justification of animal numbers appropriate for each type of study. These guidelines are intended to provide direction - any given study may not fall neatly into one of these five categories. **Select the appropriate box(es)** below and supply a narrative explanation that will clearly explain your rationale and justification for the number of animals proposed for your activity:

☐ 1. **Teaching Protocols:** (Animal numbers are determined by a specified student-to-animal ratio, which must be explained in the justification narrative. Animal numbers should be minimized to the fullest extent possible without sacrificing the quality of the hands-on teaching experience for students).

☐ 2. **Tissue Harvest Required for *In-vitro* Work and / or Antibody Production:** (Animal numbers are determined by the amount of tissue required and the number of individual animals needed to provide the appropriate amount of tissue, antibodies, etc. A detailed explanation of how the required number of animals was determined must be included in the justification narrative).

☐ 3. **Exploratory Study Requiring No Statistical Analysis - Qualitative**: **(**use of live animals to demonstrate success or failure of a desired goal, such as the production of transgenic mice): Animal numbers are justified based on the probability of success of the experimental procedure; a detailed explanation of how that probability was determined must be included in the narrative).

☐ 4. **Pilot Studies**: (Animal numbers are determined by the investigator's experience and personal judgment, and are typically small. Data collected in pilot studies are generally used to determine statistically relevant sample size calculations for future experiments).

☑ 5. **Studies Requiring Inferential Statistical Analysis**: (If possible, animal numbers are determined by statistical power analysis; the justification statement must include the specific test, i.e., ANOVA, student t-test, chi square, etc., used to determine sample size. Alternatively, minimum numbers of animals may be determined based on pertinent literature for comparable studies in which the desired effect sizes were shown to be statistically significant).

☑ a. **Statistical Test:**

Sample size calculation was performed to identify necessary sample size to distinguish between treatment groups accounting for repeated measures over time. Type 1 error at 5% and type 2 error rate set at 20% (80% power). Calculations were performed for differences in percent of T-cells producing interferon, PCR CT values, and ELISA antibody levels. The largest sample size required was to detect differences in T-cell interferon production, requiring 6 dogs in each group to detect the expected differences between vaccinates and controls over time.

☐ b. **Literature Reference:**

1. Reference- (provide specific reference(s) for numbers justification)

2. Narrative Justification- (provide a succinct justification / rationale for using the reference(s) to determine the numbers proposed in the activity)

☐ **6**. **Other**: (This applies if your activity does not fit into one of the other categories. If you check this option, you must provide a detailed and defendable description of the rationale for the number of animals proposed for your activity).

VI. HUMANE CONSIDERATIONS:

A. **Pain Category** (for your proposal, please estimate the number of animals in each applicable pain category below to the best of your knowledge - it may be appropriate to list animals in more than one pain category, i.e. controls in Cat. C, infected animals in Cat. D or E. If more than one species is requested, provide pain category estimates on all species requested. We are required to report this animal use and pain category information annually to the USDA).

USDA Pain and/or Distress Category

Please estimate the number of animals in your proposed activity that would fall into one or more of the following three pain and/or distress categories. It is common to have animals listed in more than one category - for example, an uninfected control versus a challenge group. The cumulative total number for the three Pain Categories should equal the total number of animals requested in Section V.D.

SPECIES #1 (common name): Dog

Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	108
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	

(If you are using more than one species in this activity, also complete the following section)

SPECIES #2 (common name):

Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	

SPECIES #3 (common name):

Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	

If more species are used, please list them on an attached sheet.

* List animals in USDA Pain Category B that are being bred, conditioned or held for use.

* List animals in USDA Pain Category C that will undergo no activity that will produce pain and/or distress, or procedures similar to those that might routinely be performed on humans by a physician without provision of anesthesia or analgesia, i.e. injections, phlebotomy, ear tagging, etc. If you only listed animals in category B or C, you may skip Sections VI.B-F below and resume with Section VI.G.

** List animals in USDA Pain Category D that will undergo procedures where pain-alleviating methods are used, such as anesthesia, analgesia. Surgical patients would fall into this category, even if the procedure were terminal. If you placed animals in Category D or E, you must carefully complete Section VI. B-D below

*** List animals in USDA Pain Category E that will experience unalleviated pain and/or distress. This should be considered only when the use of a pain alleviating strategy would seriously compromise the validity of the study, and/or no other option is available or possible. If you place animals in Category D or E, you must carefully complete Section VI.B-D below.

The IACUC approves protocols for a period of 3 years, so the number(s) listed here should represent the **TOTAL** number of animals requested for a project up to a three-year period- and not simply reflect annual usage projections.

VI.B. Alternatives to Painful Procedures (If you have animals listed in Pain Category D or E above, you must provide the following information. The Animal Welfare Act requires that you provide a narrative description of methods used and sources searched to ensure that you have verified that alternatives are not available to prevent unnecessary pain and distress. The Animal Welfare Information Center (AWIC) has a site that gives tips for performing this search https://www.nal.usda.gov/awic/alternatives-literature-searching. Gayle Willard, Dir, Vet Med Library is the IACUC consultant. Please contact her if you need assistance. Phone 2-6006; email: gwillard@vet.ksu.edu).

1. **Date of literature search** (should be within the last month): 09/16/2020
2. **Search at least two appropriate databases and provide the years of coverage** (i.e., PubMed (1950/current), CAB (1910/present). A list of databases is available online at https://guides.lib.k-state.edu/sb.php?subject_id=38563:

1) Pubmed (1950-present)

2) CAB Direct (1920-current)

3)

3. **Keywords/Search Strategy:**

Replacement: cell culture, in vitro, audiovisual, alternative, digital, computer, virtual, tissue culture, alternatives, simulation, dog, dogs, canis familiaris, Ehrlichia chaffeensis

Refinement: Pain and/or distress of animals will be alleviated through monitoring of clinical signs in accordance with animal monitoring plan.

4. **Concise Narrative:**

Replacement: The search with key words for replacing dogs with alternate systems did not result in a model system that can fulfill the goals of the propose project goals.

Refinement: Refinements will be used per AV consult with Dr. Marlow.

VI.C. Painful Procedure Justification (How do you plan to minimize unnecessary pain and/or distress? You must provide strong justification for having animals in Category D or E above):

Typically, *E. chaffeensis* infections do not result in fatal outcomes, nor do animals develop sever clinical illnesses. If the animal does develop an illness for some unknown reason, a CMG veterinarian will be consulted regarding the options of appropriate pain alleviation option and if required euthanasia may be recommended.

Refinement: To prevent unnecessary pain and distress, benadryl will be administered 30 min prior to *E. chaffeensis* infection. This is an established procedure we have been following over the years.

VI.D. Attending Veterinarian Consultation: ☑ **Yes** ☐ **No**

Name: Dr. Denver Marlow Date of Consult: 08/20/2020

If you have animals listed in Pain Category D or E in paragraph VI.A. above, the AWA requires that you formally consult with the IACUC attending veterinarian (AV) or his designee on all aspects of pain and / or distress management. This must be done prior to submission of the proposal to the IACUC / URCO. (Reference IACUC Guideline #22 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/22.html. To facilitate scheduling the AV consultation, please contact Ms. Shirley Whitney in the CMG office (103 Coles Hall, 532-5640, or swhitney@vet.k-state.edu) *Important note: the AV consult is not the IACUC review of your proposal. Please understand that the IACUC committee is autonomous and members will likely ask different questions they deem appropriate during the actual committee review.

VI.E. Prolonged Restraint: ☐ **Yes** ☑ **No** (Describe and justify any plans for prolonged restraint >15 min. Reference IACUC Guideline #2 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/2.html)

VI.F. Pain or Distress Alleviation - Will you be administering drugs or compounds for sedation, anesthesia or analgesia as a premedication or for anesthetic induction or maintenance? ☑ **Yes** ☐ **No** **(If "YES", all animals receiving the drug or compound will need to be placed in USDA Pain Category D.)**

1. List all drugs or compounds being used for sedation, anesthetic or analgesia during the course of your procedure. Included drug/compound name, dosage, route and frequency.

Drug/Compound	Dosage	Route	Frequency
Dexmedetomidine hydrochloride (Dexdormitor) (Note: The mcg/kg dosage decreases as body weight increases.)	23-25 mcg/kg (0.4-0.5 ml/dog) or 16.8-19.6 mcg/kg (0.3-0.4 ml/dog)	Intramuscular (IM) or Intravenous (IV)	Once prior to euthanasia to draw the blood via cardiac puncture

2. How will you monitor the animal to ensure the animal is properly anesthetized?

Animal will be monitored for sedation by checking blink, pupillary and muscular reflexes. Lowered respiratory and heart rate as well as ventral recumbency will also be considered as a sign of proper sedation.

VI.G. Surgery ☐ **Yes** ☑ **No** If No, please skip to the next section, VI.H. Animal Monitoring.

(Reference IACUC guidelines #4 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/4.html, #10 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/10.html)

1. **Procedure** (Describe surgical procedures planned)

2. **Location** (Where is the surgical procedure to be performed?)

3. **Surgeon/Qualifications** (Who will perform procedures? List their training and qualifications.)

4. **Multiple Survival Surgery Procedures** ☐ **Yes** ☐ **No** (If yes, please provide justification)

(Reference IACUC guideline #7 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/7.html)

5. **Non-Survival Surgery Procedures** ☐ **Yes** ☐ **No**

VI.H. Animal Monitoring - In order to evaluate potential for pain and/or distress, the KSU IACUC requires an approved plan of how pain or distress will be minimized and documentation of how observations of animals will be recorded. All procedures performed upon an animal should be listed on an **Animal Monitoring Plan (AMP)** form. The AMP form along with the **Animal Observation Record (AOR)** detail how you will observe your animals and what actions you will take in order to minimize pain or distress associated with your research project. Examples of procedures needing an AMP include surgical procedure, animals that undergo anesthesia, animals experimentally infected with an infectious disease, experimental vaccination, or animals inoculated with potential tumor forming cells. Exceptions to the use of the AMP and AOR would be simple procedures with minimal physiological effect upon the animal, examples of which include blood collection, or injection of therapeutic drugs.

If an AMP is included in your approved IACUC document, it is your responsibility as the PI to assure that the AMP activities will be used as described in the approved protocol. It it is your responsibility to be able to provide documentation for the activities called for in the AMP.

1. Does this protocol require the use of the AMP and AOR? ☑ **Yes** ☐ **No**
(Checking "YES" will make the AMP form appear on the next page)

2. Is an AMP completed? ☑ **Yes** ☐ **No**

3. Indicate where the AMP will be kept (i.e. animal room posted on wall, lab or barn office).

AMP will be posted outside the animal room.

Animal Monitoring Plan

Protocol #:	[redacted]	PI:	Roman Ganta	PI Contact #:	2-4612 (O): [redacted]
Animal/Group ID:		Species:	Dog	Animal Location:	CMG assigned
Procedure:	In vivo screening of E. chafffeensis mutants in dogs and ticks by intravenous administration of mutants and by tick acquisition feeding			Date of Procedure:	

I. Post-Procedure Care (if applicable)

A. List all drugs/medications to be given following the procedure (include name, dose, route, and frequency)

Drug/Medications	Dose	Route	Frequency

B. List all other care to be provided following the procedure and note frequency.

Post-Procedure Care	Frequency

II. Observations

A. Observation Frequency: Animals will be observed daily for first two weeks and on Monday, Wednesday and Friday thereafter. Observation frequency will be increased if an animal is sick.

B. When will the animal be returned to its cage/pen:

If animals are healthy, they will return immediately to pens. On the contrary, if an animal is sick then we will discuss with AV consultant and will follow as per his/her recommendation for returning to a pen.

C. List the parameters to be monitored, criteria to monitor for and directions for recording, and the appropriate action to be taken if necessary.

Parameter	Monitoring Criteria	Intervention
Temperature	Daily monitoring for first two weeks and in addition when an animal exhibits visible changes in its activity	If an animal has temperature above 104 F, CMG veterinarian will be contacted for supporting care.
CBC	Once a week	If an animal is severely anemic (as judged from the CBC analysis where PCV value is less than 35%) or significant change in differential blood counts, we will consult with the assigned CMG veterinarian to initiate appropriate course of action.
Animal Behaviour	depressed, lethargic for more than 24 hrs	CMG veterinarian will be contacted for appropriate action.
Appetite	daily monitoring for the eating pattern and assess changes in the apetite	CMG veterinarian will be contacted for appropriate action.
Tick attachment site	Swelling, redness rashes, edema and abscess	CMG veterinarian will be contacted for appropriate action.

III. Contact Information:

	Name	Telephone Number
PI	Roman Ganta	785-532-4612 (O) [redacted]
Co-Investigator		
Co-Investigator		
Veterinarian	Sally Olson	785-410-7513

In the event that the investigators or the responsible veterinarian cannot be reached or if you have concerns about an animal's care, please contact the KSU Attending Veterinarian (785-532-5648).

VI.I. Animal Manipulations:

1. **List all other drugs and compounds** that you will be administering other than those listed above in Pain or Distress Alleviation (Section F), on the Animal Monitoring Plan (Section H) or in Euthanasia (Section J.8). Include drug, dosage, route and frequency. **If any drugs/compounds are to be stored at the Veterinary Health Center Pharmacy, please consult Landa Colvin-Marion, (785)532-4127 lcolvin@k-state.edu, prior to ordering to ensure adequate storage space/requirements.**

Drug/Compound	Dosage	Route	Frequency
Ehrlichia chaffeensis mutants	1X10^8 bacteria/ml	I/V	Once for each experiment
Diphenhydramine (Benadryl)	1 mg/lb body weight	oral	Prior to i/v infection

2. If any of the above drugs/compounds are to be prepared in your lab, please explain the preparation and storage process.

3. **List any rooms where procedures with animals are done (excluding housing and surgery).** Locations for procedures such as behavior testing, treadmill training, blood draws, injections, gavage, etc. should be listed in this chart. If procedures are performed within CMG facilities, the "CMG assigned".

Building/Room Number	Procedure

4. **Biosamples:** ☑ **Yes** ☐ **No** (list type & amount, i.e., phlebotomy, minor biopsies, ascitic fluids, etc.)

Approximately 10 ml blood will be collected twice weekly in EDTA from cephalic veins and or in sodium heparin or red tubes using vaccutainer tubes with 19-22G needles for the first two weeks for all three experiments and once a week there after. The blood will be collected from jugular vein or anterior cephalic or lateral cephalic vein. Postmortem tissue sampling will include the collection of blood (about 50 ml), spleen, liver, lymph nodes, lung, and bone marrow and they will be used for final detailed assessment of infection status. A total of a maximum of 11 blood samples from each animal will be collected.

5. **Tissue Sharing:** ☐ **Yes** ☑ **No** (detail any tissue sharing you plan with other investigators)

6. **Other Procedures:** (list any other procedures you might perform on animals in this project)

7. **Adjuvants:** ☐ **Yes** ☑ **No** (explain any adjuvant use. Reference IACUC guideline #12 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/12.html)

8. **Chemical Grade Drugs:** ☐ **Yes** ☑ **No** (If you plan to use a chemical grade please list and provide a scientific explanation for its use; Reference IACUC guideline # 19 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/19.html)

VI. J. Veterinary Care:

1. **Animal Housing:** (Provide specific information on where the animals will be housed for your activity.)
PLEASE INCLUDE ROOM NUMBER IF KNOWN

[redacted]

2. **Social/Paired Housing:** (Social animals should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility. "The Guide" 8th Edition):

☑ **Yes** ☐ **No** My animals will be housed in stable pairs or compatible groups?

If no, please provide an adequate justification for an exception to this guidance.

3. **Special Husbandry Considerations:** (Animals will be housed in designated animal rooms/areas, unless approved by the IACUC. Detail special husbandry requirements, i.e. special diets, micro-isolators, etc.):

Dogs with tick chambers will need to be housed individually for 7 days.

4. **Animal Surveillance:** (Who observes the animals daily for health problems?)

The PI and qualified research staff.

5. **Veterinary Clinical Care:** (Who will you contact if there is a health problem requiring veterinary care?)

CMG veterinarians

6. **Wire Bottom Rodent Caging:** If you are using rodents, do you propose to house them in wire-bottom cages?

☐ **Yes** ☐ **No** (If yes, you must explain the rationale for the use of wire bottom cages scientifically. See IACUC Guideline #14 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/14.html)

☑ **N/A**

7. **Study Endpoint** (Experimental studies may involve procedures that cause clinical symptoms or morbidity in animals. The IACUC must consider the selection of the most appropriate endpoint(s). This requires careful consideration of the scientific requirements of the study, expected and possible adverse effects research animals may experience (pain, distress, illness, etc.), the most likely time course and progression of those adverse effects, and the earliest most predictive indicators of present or impending adverse effects. Optimally, studies are terminated when animals begin to exhibit clinical signs of disease if this endpoint is compatible with meeting the research objectives. Such endpoints are preferable to death or moribundity as endpoints since they minimize pain and distress. **The use of death of the animal as an endpoint is strongly discouraged and must be justified to the IACUC - Reference IACUC guideline # 13 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/13.html**. Please describe the endpoint of your study):

After about 60 days of study all animals used in the three experiments will be euthanized .

8. **Euthanasia:** (Reference the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, link available on the KSU IACUC or the AVMA website, **https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx**)

Will animals be euthanized as a part of your protocol? ☐ **Yes** ☐ **No**

i. **Method** (include drug, dosage, and route)

Before euthanasia, animals will be deeply sedated with Dexmedetomidine hydrocholride (23-25 mcg/kg) and approximately 50ml blood will be collected by cardiac puncture. Euthanasia will be performed in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA). Specifically, commercial euthanasia solution, Fatal-Plus®, of volume 0.22 ml/kg (86 mg/kg of pentobarbital) to effect will be administered intracardially after the terminal bleed.

ii. **Name of person(s) responsible for performing the euthanasia.**

CMG veterinarian or qualified research staff.

9. **Animal Disposition** (what is your plan for the animals after the study is over?)

☑ **Euthanasia** ☐ **Adoption** ☐ **Long-term holding**

☐ **Transfer to another investigator with approved or pending protocol.**

Name:

☐ **Other**

VII. Investigator & Technician Qualifications/Training: List all persons involved in your activity below - excluding CMG and LACS personnel - and their professional training. **Include years of experience with the specific species and procedures listed in this proposal.**
Contact the University Research Compliance Office, 532-3224 for information or guidance on animal care and use training

Name	Training and experience with the species and procedures listed in this protocol
Roman R. Ganta	PhD; worked with mice for about three years, deer for two years and dogs for about 5 years
Swetha Madesh	M.S., worked with dogs for 6 months
Deborah C. Jaworski	PhD; worked with dogs for 5 years; worked with other animal models in excess of 5 years (sheep, deer, cattle, rats, mice, rabbits)
Jonathan Ferm	B.Sc.,Trained veterinary technician, RVT, worked with dogs for 3 years
Paidashe Hove	Ph D; worked with dogs for 2 years; large animals for 5 years
Ashley Thackrah	M.S., worked with sheep, deer, pigs, mice and chickens
Dominica Genda	DVM, MS worked with dogs for 2 years; large animals for 5 years
Kimia Alizadeh	DVM, worked with dogs for 2 years; large animals for 5 years
Cheyenne Knox	B.S., Worked with dogs for 6 months
Shawna Fitzwater	M.S.; worked at vet clinic for 4 years working with small and large animals, including with dogs.

The IACUC is required to review and approve changes in personnel for research or teaching involving animals. **Consequently, you must inform the IACUC (via protocol modification) of any changes in animal care research personnel that may occur in your activity. Additionally, you must ensure that new personnel involved in your activity are qualified, have completed the mandatory animal care and use training, and are enrolled in the occupational health and safety program.

☑ **Yes** ☐ **No** **Will personnel be trained in humane handling of this species?**

☑ **Yes** ☐ **No** **Are all personnel enrolled in the KSU Animal Worker Occupational Health and Safety Program?**
(If no, forms can be downloaded from http://www.k-state.edu/research/comply/iacuc/ohsp or you may contact the University Research Compliance Office (2-3224) for information.)

☐ **Yes** ☑ **No** **Will you need animals for protocol-related training purposes, i.e., experimental or surgical technique development or refinement, etc.?** If yes, please specify the technique or procedure to be performed during training (you may reference detailed description in another section of the proposal if appropriate):

Number of animals required to accomplish the proposed training (be sure to include the number of animals requested for training purposes in the total number of animals listed in Section V.D., and Section VI.A.):

Please indicate how training is/will be accomplished:

☑ Yes ☐ No **Training and/or orientation with P.I., CMG or LACS personnel**

☐ Yes ☑ No **Instruction by supervising animal caretaker**

☐ Yes ☑ No **Viewing of instructional videos**

☐ Yes ☑ No **Other (please specify)**

☑ Yes ☐ No **Individual Technical Procedure Training Form.**

if you marked no, explain below how you are going to document training or technical competence for personnel to perform the procedure(s) proposed.

If you are proposing to use a technical, manipulative, or invasive procedure on animals as part of your activity, it is a requirement that you document the competence of your staff to perform the proposed procedure. Documentation of training is necessary for all personnel for specific animal use procedures such as handling, stomach tubing, euthanasia, injections, biopsy, phlebotomy, restraint, etc. This formal training documentation should be maintained in the laboratory or close by and be readily available for IACUC, USDA, AAALAC, OLAW and research compliance review as appropriate. It is the PI's responsibility to ensure that adequate training is performed, and documented. If you need assistance with training for technical procedures, contact the attending veterinarian (532-5648) or the university veterinarian (532-3224) for advice or assistance.

VIII. Hazardous or Potentially Hazardous Material Use: (explain if you are using hazardous or potentially hazardous materials in your study)

1. ****Biological, Infectious or Toxic agents** ☐ No ☑ Yes (list)

Ehrlichia chaffeensis and nymphal stage *Amblyomma americanum* ticks

2. ****Recombinant or synthetic nucleic acid molecules** ☑ No ☐ Yes (list)

3. **Hazardous chemicals** ☑ No ☐ Yes (list)

4. **Radioisotopes** ☑ No ☐ Yes (list)

5. **Other (example: Nanoparticles)** ☑ No ☐ Yes (list)

6. ****Select Agents:** Are you using or planning to use agents listed in the Federal Select Agent Program. (http://www.selectagents.gov/SelectAgentsandToxinsList.html)?

☑ No ☐ Yes (list)

The Federal Select Agent Program (www.selectagents.gov), a joint program of the Centers for Disease Control and Prevention (CDC), and the USDA Animal and Plant Health Inspection Service (APHIS), oversees the activities of possession, use and transfer of biological agents and toxins that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products.

If you plan to use or are using any of the viruses, bacteria, fungi, rickettsial agents, or toxins on the select agent list, please contact the K-State Responsible Official for select agent use at the Biosecurity Research Institute (785-532-3248), or the URCO (785-532-3224) for information.

(If "yes" you must have a Registration Document from the Institutional Biosafety Committee)**

IBC Registration Document # [redacted] **Approval Date**

IX. DEA Controlled Substances: Guide For the Care and Use of Laboratory Animals "All those involved in animal care and use must comply with federal laws and regulations regarding human and veterinary drugs and treatments."

☒ Yes ☐ No Are you using any DEA controlled substances in this protocol? If "Yes" please list them below.

Fatal-Plus (Pentobarbital) 390mg/mL

Name of DEA Registration Certificate Holder Arathy DSNair

X. Extramural Funding: (It is critical that animal care and use procedures detailed in the IACUC protocol are consistent with external funding proposals documents. Discrepancies between the two documents in animal care and use procedures could jeopardize individual and/or institutional funding and compliance. If you make changes, or they are required by the IACUC, it is your responsibility to ensure that grant or funding agencies are informed.)

☑ **Yes** ☐ **No** All animal care and use procedures described in this proposal are consistent with those described in external funding applications/documents. If no is checked, please contact the URCO (532-3224).

☐ **N/A**

XI. Clinical Research:

☐ **Yes** ☑ **No** Does this protocol involve client owned animals?

If yes, have you attached a copy of the client consent form to be used? ☐ **Yes** ☐ **No**

Please visit: URCO - IACUC - Resources - Protocol Development https://www.k-state.edu/comply/iacuc/resources/index.html For client consent form examples/templates.

If the protocol is associated with the veterinary health center, please consult Kris Richardson, (785)532-3046 krichardson@vet.k-state.edu, **prior to submitting a client consent form to the IACUC committee for review.**

Date of Consult with Kris Richardson

XII. USDA Regulated Activities: (Is your activity regulated by provisions of the Animal Welfare Act?) Contact the URCO or the attending veterinarian if you need clarification.

Regulated animals would include: - Any live or dead dog, cat, monkey, guinea pig, hamster, rabbit, or warm-blooded animal used for biomedical research, teaching, testing, experimentation, or exhibition purposes. Exemptions to this definition are listed below.

Exempt or non-USDA regulated animals would include: (1) lab rats and mice (*Mus* / *Rattus*) bred for use in research, (2) birds, (3) horses not used for (biomedical) research purposes, and (4) other farm animals such as, livestock or poultry, used or intended for use as food or fiber, or improving animal nutrition, breeding, management, production efficiency, or for improving food or fiber quality.

☑ **Yes -** My activity involves species **COVERED** by the definition of animal in the Animal Welfare Act.

☐ **No -** My activity involves animals that are **EXEMPT** from coverage by the USDA

☐ **Both** - My activity involves both covered and exempt species.

XIII. Wildlife or Field Investigation:

☐ **Yes** ☑ **No** Does your activity involve the use or observation of nondomesticated vertebrate species under field conditions?

If "Yes," please answer the following:

☐ **Yes** ☐ **No** Does your wildlife field activity require any international, federal, state or local permits? If "Yes" please provide copies of permits.

☐ **Yes** ☐ **No** Are you using any relevant professional society guidelines that are available for your wildlife field activity?

If "Yes," please list:

Online Required Training

TRAINING REQUIREMENTS HAVE RECENTLY CHANGED

The IACUC requires mandatory training prior to protocol approval. Training is now offered through the Collaborative Institutional Training Initiative (CITI) Program. Instructions to register and access training are found on the URCO website: http://www.k-state.edu/research/comply/

Use the check boxes below to select the training courses that apply to this protocol. If you have any questions about training, contact URCO at comply@k-state.edu, or (785) 532-3224.

Mandatory Training

Required for all Principal Investigators, research staff and students

☒ Responsible Conduct of Research ☒ Working with the IACUC

Required (Provost-mandated) for all full time K-State employees

☒ Export Compliance

Species-specific training (check all that apply to this protocol)

☐ Swine ☐ Cattle ☐ Rat ☐ Mouse ☐ Guinea Pig ☐ Hamster ☐ Ferret

☑ Dog ☐ Cat ☐ Horse ☐ Gerbil ☐ Sheep or Goat ☐ Rabbit ☐ Zebrafish

☐ Fish (except zebrafish) ☐ Amphibians ☐ Wildlife (except fish) ☐ Farm Animals or Agricultural Animals

Required procedure-specific training (check all that apply to this protocol)

☐ Survival Surgery

☐ Rat or Mouse, Category D or E procedures

☐ Antibody Production

All new personnel or personnel with expired training are required to register for CITI and take the new training requirements. If you previously completed online IACUC modules, your training status will remain current until it expires. URCO will verify training from the previous system as well as the new system prior to approval of any protocol.

POST APPROVAL MONITORING: The URCO has a Post-Approval Monitoring (PAM) program to help assure that animal care and use activities are performed in accordance with provisions or procedures approved by the IACUC. Accordingly, the URCO staff will arrange PAM visits as appropriate to assess compliance with approved activities.

INVESTIGATOR ASSURANCE FOR THE HUMANE CARE AND USE OF ANIMALS FOR TEACHING AND RESEARCH

(Print this page separately because it requires a signature by the PI.)

P.I. Name: Dr. Roman Ganta

Title of Project: Vector and host contributions to Ehrlichia chaffeensis gene expression [redacted]

XIII. ASSURANCES: As the Principal Investigator on this protocol, I provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, and in accordance with applicable laws, regulations, and guidelines. Any deviation or modification from the procedures detailed herein, must receive prior approval from the Institutional Animal Care and Use Committee (IACUC).

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that there has been an adequate evaluation of the experimental design or strategy of this proposal, and that the minimum number of animals needed for scientific validity are used.

D. Oversight: All experiments, surgeries, or manipulations involving live animals will be performed under my supervision or that of another qualified individual. In procedures involving USDA Pain Category D or USDA Pain Category E, I have consulted with the attending veterinarian on minimizing pain and/or distress.

E. Biohazard\Safety: I assure that in planning this proposal, I have made the proper consideration regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant DNA issues, etc. Additionally, personnel on my study with contact with animals are enrolled in the Animal Worker Occupational Health and Safety Program.

F. Training: I assure that personnel performing animal procedures\manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures\manipulations. Inexperienced personnel will be properly trained and/or supervised. Additionally, I understand that I must maintain documentation of appropriate animal care and use training for personnel involved in my study.

G. Adverse Event Notification: In compliance with provisions of both the "Ag" and "ILAR Guide," I assure that I will notify the IACUC Attending Veterinarian (Dr. Marlow) if there is a significant unanticipated adverse event during the execution of my activity. This would include unexpectedly high levels of mortality or development of a new disease condition that affects the health and / or welfare of the animals, etc.

H. Extramural Funding: If funded by an extramural source, I assure that this application accurately reflects all procedures involving laboratory animal subjects as described in the proposal to the funding agency. (standards are the same, regardless of funding sources).

I. Study Duration: I understand that proposals are approved for 3 years. I also understand that as subsequent annual reviews are conducted, it is my responsibility to provide timely and accurate annual review information when requested, to include notification of the IACUC and the University Research Compliance Office (URCO) when my study is completed.

You may sign this form using a digital signature. DO NOT sign the form until it has been completed.
You cannot edit the form entries once the form has been digitally signed. If you are making revisions to a previously signed form, right-click the digital signature and select Clear to remove the signature (this can only be done by the person who originally digitally signed the form). Forms that have not been signed will not be accepted.

P.I. Signature: [redacted] Date: 8/13/2021

FOR OFFICE USE ONLY: IACUC Protocol #[redacted] Application Received: 2/18/19 Routed: 2/18/19 Training Complete:
Deadline to Request Full Committee Review: Covered Species? Yes Exempt Species? No Both? No IBC #

KANSAS STATE UNIVERSITY
University Research Compliance Office

Institutional Animal Care and Use Committee (IACUC)
Application for Approval Form

Please send your completed application to comply@k-state.edu

Version: Last Updated: 06/13/2016

ADMINISTRATIVE INFORMATION:

Responsible Individual/PI: Roman R. Ganta

Responsible Graduate Student (if applicable):

Title of Project/Course: Vector and host contributions to *Ehrlichia chaffeensis* gene expression

Species/ Strain to be used: dog

Type of Application: (check one box) ☑ New ☐ Addendum/Modification (complete modification block below)

Category: (check one box) ☐ Teaching ☐ Testing ☑ Research ☐ Other (if other, describe)

Funding Source: ☑ PHS/NIH ☐ Other Federal Agency ☐ State ☐ Other

Principal Investigator: Roman R. Gantra Degree/Title: M.S., Ph.D.

Department: Diagnostic Medicine/Pathobiology Campus Phone: 532-4612

Campus Address: 336 Coles Hall, 1800 Denison Ave, KSU-CVM

E-mail: rganta@vet.ksu.edu Alternate phone :

Co-Principal Investigators:

Name: Dept: Degree/Title:

Name: Dept: Degree/Title:

MODIFICATION:

Is this a modification of an approved protocol? ☑ **Yes** ☐ **No** **If yes, please comply with the following:**

If you are requesting a modification or a change to an IACUC approved protocol, please provide a concise description of all of the changes that you are proposing in the following block. Additionally, please highlight or bold the proposed changes in the body of the protocol where appropriate, so that it is clearly discernible to the IACUC reviewers what and where the proposed changes are. This will greatly help the committee and facilitate the review.

I request the following minor modification:

Please delete the following members:

1) Andy Alhassan
2) Katie Reif

Please add the following members:

1) Arathy Nair
2) Deborah Jaworski
3) Paidashe Hove

I. NON-TECHNICAL SYNOPSIS (Please provide a brief narrative description of proposal. This should typically be less than 75 words and be easily understood by nonscientists, e.g. `*We propose to test the effectiveness of a new class of anti-inflammatory drugs against arthritis that develops in the hips of dogs affected by congenital hip dysplasia*'):

Goals of this protocol are to evaluate tick transmitted bacterial (Ehrlichia chaffeensis) mutants to discover proteins involved in causing the disease, human and canine monocytic ehrlichiosis, using the dog infection model. Dog studies are preferred as the pathogen causes disease in this host similar to people. The study outcomes will be important in developing disease preventing strategies in people and dogs.

II. BACKGROUND (concise narrative review of the literature and basis for the study):

Dog was chosen as the infection model for the proposed experiments because it is an incidental host in acquiring *E. chaffeensis* similar to humans. Moreover, our recent experimental studies demonstrated that dog serves as an excellent infection model, where the pathogen infection persists in this animal and without causing severe disease. In particular, our recent experimental infection study demonstrated that dogs develop only mild fever (rise in only up to 1.5ºC body temperature), while maintaining a persistent infection with detectable hematological changes, host response and pathology.

This application is the replacement of our recently expired IACUC application # [redacted]; the protocol will allow us to complete the experiments that were part of the protocol #[redacted]. The protocol #[redacted] had two goals; 1) vaccine studies and 2) assessment of 200 mutants of *E. chaffeensis*. Vaccine studies were completed and the mutant assessment studies were yet to be completed. Vaccine studies were carried out on three attenuated mutants developed several years ago following the discovery of attenuated mutants following screening 9 mutants in dogs and deer. The previous studies of screening 9 mutants led to the establishment of all the needed protocols in place which will be used in the current application. However, the previous study was not sufficient to learn all of the important genes involved in causing pathogenesis by *E. chaffeensis*. The current study, thus, extends to characterize up to 200 gene mutants. The current study will use the same protocols as before.

The recently expired protocol (#[redacted]) was part of the funded NIH R01 grant proposal and it was expired due to limitation of the IACUC policy, while the grant is still active (five year grant). This new IACUC protocol application goals are the same as second objective of the expired protocol; i.e., to request approval to complete the remaining unfinished portion of the project (only one major objective left).

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]. E. chaffeensis, E. ewingii, E. canis, and A.

phagocytophilum also cause infections and diseases in dogs. Tick-transmitted pathogens have evolved strategies to persist in both tick and vertebrate hosts in order to successfully complete their infectious cycle.

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 [11-13]. 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 [14]. Furthermore, the host response differed considerably from *E. chaffeensis* originating from tick and vertebrate host environments [14]. 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.

We recently performed mutational analysis and demonstrated that mutations in three different genes (Ech_0230, Ech_0379 and Ech_0660) of *E. chaffeensis* caused attenuated growth of the organism in vivo [15]. These data formed the basis for our funded NIH-R01 grant application having the following three 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.

Specific aims 2 and 3 involve the use of experimental infection studies in dogs. We already completed the goals of aim 2 as part of our expired IACUC application # [redacted]. This application will focus on the goals of aim 3.

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1. Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, et al. (2001) Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophila. IntJSystEvolMicrobiol 51: 2145-2165.
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Ehrlichia interactions with tick cells and macrophages. Front Biosci 14: 3259-3273 (PMID: 19273271).
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14. Ganta RR, Cheng C, Miller EC, McGuire BL, Peddireddi L, et al. (2007) Differential clearance and immune responses to tick cell-derived versus macrophage culture-derived Ehrlichia chaffeensis in mice. InfectImmun 75: 135-145(PMID: 17060466).
15. Cheng C, Nair ADS, Indukuri VV, Gong S, Felsheim RF, Jaworski D, Munderloh UG and Ganta RR.
Targeted and Random Mutagenesis of *Ehrlichia chaffeensis* for the Identification of Genes Required for In vivo Infection. PLoS Pathog. (2013) 9:e1003171. doi: 10.1371/journal.ppat.1003171.

III. LITERATURE SEARCH FOR UNNECESSARY DUPLICATION

(If your proposed activity is part of the formal veterinary teaching curriculum and is not research or testing, you may not have to perform a literature search for unnecessary duplication. If it is teaching, please go to http://awic.nal.usda.gov/ for guidance on how to address Section III. A literature search for unnecessary duplication is required for all proposed research activities using animals.)

A. Date of literature search (should be within the last month): 08/11/2017

B. Search at least two appropriate databases and provide the years of coverage (i.e., PubMed (1950/current), CAB (1972/present)). A list of databases is available online at **http://www.lib.ksu.edu/db/subject/vetmed.html**:

1) Pubmed (1950-present)

2) CABI: CAB Abstracts® and Global Health (1910-present)

3)

C. Keywords/Search Strategy:

dog, dogs, Canis familiaris, Ehrlichia chaffeensis, transmission

D. Please provide a concise narrative of the results of the searches relative to unnecessary duplication. You do not need to provide a copy of the actual search with your proposal, but it should be maintained for your records or available to the IACUC if requested. Gayle Willard, Dir, Vet Med Library is the IACUC consultant. Please contact her if you need assistance. Phone 2-6006; email: gwillard@vet.ksu.edu

Concise Narrative:

We looked at alternatives for infection studies as proposed in the study. Our detailed evaluation of the published reports did not reveal any possible alternatives to infection studies.

IV. OBJECTIVE\HYPOTHESIS (briefly state the objective of the study - and, if applicable, the hypothesis to be accepted or rejected):

The objectives of this IACUC application are to conduct animal infection experiments in support of our funded NIH R01 grant application. Specifically, we will perform in vivo screening experiments to identify genes essential for *E. chaffeensis* pathogenesis in vertebrate and tick hosts.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures (succinctly outline formal scientific plan for study):

Objective: Perform mutational analysis and *in vivo* screening to identify genes essential for the *E. chaffeensis* pathogenesis in vertebrate and tick hosts. We will generate large pools of *E. chaffeensis* transposon mutants in support of this objective. Our funding was approved to generate 200 mutant organisms. These mutants will then be screened to define the pathogenesis using the canine infection model; three experiments were proposed to accomplish this goal.

Experiment 1) 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. Tick cells will be placed on dogs and covered with sheep sox (made of Nylon Spandex for easy flexibility) (Sheepman Supply co. or something similar) by following the procedures similar to those done on deer, except that there is no need for anesthetize the dogs. For these experiments, the backs of the animals will be shaved with veterinary clippers. A custom designed tick containment chamber (modified top of Nalgene jar containing screw cap lid) will be glued to polyvinyl membrane with a center circular opening. The chamber will then be glued to animals with industrial adhesive (commercially available). The chambers have round bottom smooth surface and once glued, the chambers remain attached for several weeks until polyvinyl membrane is lifted off the skin with the hair growth. To ensure that the chambers are tightly attached, tick infestations will be performed only after about 24 h following the attachment of the chambers. In particular, we will monitor for the retainment of the chambers on the animals, as well as their firm attachment. If dogs attempt to remove the chambers, we will place Elizabethan neck collars to restrict grooming. The chambers will be covered with sheep sox. To perform the tick infestation, lids of the chambers will be unscrewed, ticks will be placed inside and the chambers will then be tightly closed with the lids and animals will be covered back with sheep sox. 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 (10 ml blood drawn twice a week from cephalic veins 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 (total dogs for this sub-experiment are 60).

Experiment 2) We will repeat the infection experiment on all those mutants identified as attenuated in vivo in the previous experiment. As in the previous experiment, 10 ml blood samples will be collected from cephalic veins and tick attachment experiment will also be performed and essentially as outlined above. We will clonally purify the mutants, randomly select pools containing 5 clones each and use them to repeat the infections in three dogs each as in the previous experiment. We expect to identify about 60

mutants from the first experiment and pools of 5 mutants each will be used per dog; thus, the total pools for this second experiment will be 12 (total dogs to be used for this experiment will be 36).

Experiment 3) 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 for this experiment). Similar to experiments 1 and 2, blood draws (10 ml each time from cephalic veins) and tick attachment experiments will be performed as outlined above 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 of all three experiments will be 126 (60+36 +30).

In all three experiments, the animals will be kept for 60 days each to monitor the mutant *E. chaffeensis* circulation in blood. Blood sampling will be done twice a week from cephalic veins (10 ml each) for the first two weeks and then once a week thereafter. Total blood draws will be 11 times per animal.

About 6 to 8 month-old dogs of the breed 'Beagle' will be used for these experiments. For convenience, we will either use all males or all females in each experimental group. The weight of each animal will be about 15 to 20 pounds. Diphenhydramine (Benadryl) (1mg/ml) will be orally administered to all animals about 30 minutes prior to inoculation with Ehrlichia. (The stock concentration to be used is 2.5 mg/ml; 6 to 8 ml per animal for 15 to 20 pound dogs.) Benadryl is administered to prevent any possible anaphylactic shock resulting from injection of organisms containing traces of serum or other animal products likely present in the culture media.

The terminal bleed (by heart puncture method) serum is the only source to collect up to 50 ml of blood-derived high titer sera from animals. We request this option to draw blood just prior to euthanasia. When possible, we will do that via cephalic or saphenous vein. We will need to sedate the dogs with Dexmedetomidine hydrochloride (Dexdormitor) 0.5mg/ml at a dose of 23-25mcg/kg (0.4-0.5 ml/dog) for IM injection or 16.8-19.6 mg/kg (0.3-0.4 ml/dog) for IV injection and draw the blood via cardiac puncture once dog is complete sedated.

B. Non-animal Alternatives Considered (were non-animal alternatives considered - why are they not used?):

It is not possible to have any non-animal alternatives for understanding the disease progress comparisons, immune responses and pathogens' persistence.

C. Animal Model and Species/Strain Justification (Explain why animals are needed for your study. Give your rationale and justification for selecting this animal model or species):

Dogs-beagle (breed); the dog is the perfect animal model for such studies because it acquires E. chaffeensis infections naturally like humans and moreover tick transmission studies can be done in this animal model similar to those likely occurring in people. Furthermore, the dog model supports the development of persistent infections. The beagle is chosen for this study because it is the most commonly reported breed for similar studies in the literature and moreover, it is easy to work with this breed. Finally, this breed of dog is commercially available for use in experimental studies.

D. Animals Requested -used in research testing or teaching (list genus and species/strain of animal model proposed)**:**

Genus and Species:	*Canis familiaris*
Total number (by species) requested: (this should correspond to the sum of the animals listed in Section VI.A. below. The IACUC approves protocols for a	126

period of 3 years, so the number(s) listed here should represent the TOTAL number of animals requested for a project up to a three-year period- and not simply reflect annual usage projections.)

Source of animals (by species):

We will use a commercial class USDA licensed dealer to obtain dogs.

E. Justification of Animal Numbers / Data: Analysis: Research, testing, and teaching activities should be designed to provide a statistically significant result with a minimum number of animals. The specific method by which the number of animals was determined must be clearly stated. Statistical techniques and/or power analysis are appropriate in most cases to maximize the usefulness of the data generated from each animal. However, the IACUC acknowledges that the basis for an appropriate justification of animal numbers depends largely on the nature of the study itself. Prior experience and expertise with the model in question may be taken into account as well, but must be carefully documented in the protocol. The cost of the animal should not be considered as the primary justification for the use of a particular species or model. Consultation with a biostatistician or use of statistical software during the design phase of the experiment may be useful. This website may be helpful in performing a power analysis: **http://statpages.org**

Five basic types of studies are listed below, along with brief general guidelines for the justification of animal numbers appropriate for each type of study. These guidelines are intended to provide direction - any given study may not fall neatly into one of these five categories. **Select the appropriate box(es)** below and supply a narrative explanation that will clearly explain your rationale and justification for the number of animals proposed for your activity:

☐ **1.** **Teaching Protocols:** (Animal numbers are determined by a specified student-to-animal ratio, which must be explained in the justification narrative. Animal numbers should be minimized to the fullest extent possible without sacrificing the quality of the hands-on teaching experience for students).

☐ **2.** **Tissue Harvest Required for *In-vitro* Work and / or Antibody Production:** (Animal numbers are determined by the amount of tissue required and the number of individual animals needed to provide the appropriate amount of tissue, antibodies, etc. A detailed explanation of how the required number of animals was determined must be included in the justification narrative).

☑ **3.** **Exploratory Study Requiring No Statistical Analysis - Qualitative**: **(**use of live animals to demonstrate success or failure of a desired goal, such as the production of transgenic mice): Animal numbers are justified based on the probability of success of the experimental procedure; a detailed explanation of how that probability was determined must be included in the narrative).

Screening of transposon mutants of *E. chaffeensis* have been successfully accomplished using three dogs or three deer in the past by us. As we will do repeated sampling of blood over two months and from three dogs each, the goals of the experiment will be accomplished well with using only three dogs per experiment.

☐ **4.** **Pilot Studies**: (Animal numbers are determined by the investigator's experience and personal judgment, and are typically small. Data collected in pilot studies are generally used to determine statistically relevant sample size calculations for future experiments).

☑ **5.** **Studies Requiring Inferential Statistical Analysis**: (If possible, animal numbers are determined by statistical power analysis; the justification statement must include the specific test, i.e., ANOVA, student t-test, chi square, etc., used to determine sample size. Alternatively, minimum numbers of animals may be determined based on pertinent literature for comparable studies in which the desired effect sizes were shown to be statistically significant).

☑ a. **Statistical Test:**

Experiments 1-3: Each experimental group to test mutants will include 3 dogs and blood samplings on these animals will be carried out over a period of 60 days. The continuous monitoring over several weeks and three dogs per group were shown to be sufficient to draw meaningful conclusions for identifying the mutants attenuated. Moreover, repeated analysis for further screening of the dogs in experiments 2 and 3 will further validate our conclusions. Our prior studies in deer and dogs demonstrated that this is a valuable approach in drawing conclusions where statistical significance can be assessed. This is a minimum number to draw meaningful and statistically significant conclusions, particularly when using out bred animals. The hematology data will be evaluated with one-way ANOVA and Tukey's multiple comparison test to compare each group at each time point to detect significant differences ($p \leq 0.05$). A t-test will be performed for Culture recovery and PCR results.

☑ **b. Literature Reference:**

1. Reference- (provide specific reference(s) for numbers justification)

Gaunt S, Beall M, Stillman B, Lorentzen L, Diniz P, Chandrashekar R, Breitschwerdt E (2010). Experimental infection and co-infection of dogs with Anaplasma platys and Ehrlichia canis: hematologic, serologic and molecular findings.Parasit Vectors. 8;3(1):33.

Eddlestone SM, Gaunt SD, Neer TM, Boudreaux CM, Gill A, Haschke E, Corstvet RE.(2007) PCR detection of Anaplasma platys in blood and tissue of dogs during acute phase of

experimental infection. Exp Parasitol. 115(2):205-10.

Rudoler N, Baneth G, Eyal O, van Straten M, Harrus S.(2012). Evaluation of an attenuated strain of *Ehrlichia canis* as a vaccine for canine monocytic ehrlichiosis. Vaccine. 31(1):226-33.

2. Narrative Justification- (provide a succinct justification / rationale for using the reference(s) to determine the numbers proposed in the activity)

☐ **6**. **Other**: (This applies if your activity does not fit into one of the other categories. If you check this option, you must provide a detailed and defendable description of the rationale for the number of animals proposed for your activity).

VI. HUMANE CONSIDERATIONS:

A. **Pain Category** (for your proposal, please estimate the number of animals in each applicable pain category below to the best of your knowledge - it may be appropriate to list animals in more than one pain category, i.e. controls in Cat. C, infected animals in Cat. D or E. If more than one species is requested, provide pain category estimates on all species requested. We are required to report this animal use and pain category information annually to the USDA).

USDA Pain and/or Distress Category

Please estimate the number of animals in your proposed activity that would fall into one or more of the following three pain and/or distress categories. It is common to have animals listed in more than one category - for example, an uninfected control versus a challenge group. The cumulative total number for the three Pain Categories should equal the total number of animals requested in Section V.D.

SPECIES #1 (common name):	Dog	
Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	126
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	

(If you are using more than one species in this activity, also complete the following section)

SPECIES #2 (common name):		
Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	
SPECIES #3 (common name):		
Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	

If more species are used, please list them on an attached sheet.

* List animals in USDA Pain Category B that are being bred, conditioned or held for use.

* List animals in USDA Pain Category C that will undergo no activity that will produce pain and/or distress, or procedures similar to those that might routinely be performed on humans by a physician without provision of anesthesia or analgesia, i.e. injections, phlebotomy, ear tagging, etc. If you only listed animals in category B or C, you may skip Sections VI.B-F below and resume with Section VI.G.

** List animals in USDA Pain Category D that will undergo procedures where pain-alleviating methods are used, such as anesthesia, analgesia. Surgical patients would fall into this category, even if the procedure were terminal. If you placed animals in Category D or E, you must carefully complete Section VI. B-D below

*** List animals in USDA Pain Category E that will experience unalleviated pain and/or distress. This should be considered only when the use of a pain alleviating strategy would seriously compromise the validity of the study, and/or no other option is available or possible. If you place animals in Category D or E, you must carefully complete Section VI.B-D below.

The IACUC approves protocols for a period of 3 years, so the number(s) listed here should represent the **TOTAL** number of animals requested for a project up to a three-year period- and not simply reflect annual usage projections.

B. **Alternatives to Painful Procedures** (If you have animals listed in Pain Category D or E above, you must provide the following information. The Animal Welfare Act requires that you provide a narrative description of methods used and sources searched to ensure that you have verified that alternatives are not available to prevent unnecessary pain and distress. The Animal Welfare Information Center (AWIC) has a site that gives tips for performing this search http://www.nal.usda.gov/awic/alternatives/tips.htm. Gayle Willard, Dir, Vet Med Library is the IACUC consultant. Please contact her if you need assistance. Phone 2-6006; email: gwillard@vet.ksu.edu).

1. **Date of literature search** (should be within the last month): 08/11/2017

2. **Search at least two appropriate databases and provide the years of coverage** (i.e., PubMed (1950/current), CAB (1972/present). A list of databases is available online at **http://www.lib.ksu.edu/db/subject/vetmed.html**:

1) PubMed (1950- present)

2) CABI (1910-present)

3)

3. **Keywords/Search Strategy:**

Keywords for replacement cell culture, in vitro, audiovisual, alternative, alternatives, simulation, dog, dogs, canis familiaris, Ehrlichia chaffeensis

4. **Concise Narrative:**

The search with key words for replacing dogs with alternate systems did not result in a model system that can fulfill the goals of the propose project goals.

C. **Painful Procedure Justification** (How do you plan to minimize unnecessary pain and/or distress? You must provide strong justification for having animals in Category D or E above):

Typically, Ehrlichia chaffeensis infections do not result in fatal outcomes, nor do animals develop sever clinical illnesses. If the animal does develop an illness for some unknown reason, a CMG veterinarian will be consulted regarding the options of appropriate pain alleviation option and if required euthanasia may be recommended.

Refinement: To prevent unnecessary pain and distress, benydral will be administered 30 min prior to E. chaffeensis infection. This is a common procedure we had followed in the past and we will continue the same in the future experiments proposed as part of this application. This was already discussed with Dr. Denver Marlow, CMG director and another colleague, Dr. Sally Olsen.

D. **Attending Veterinarian Consultation:** ☑ **Yes** ☐ **No**

Name: Dr. Denver Marlow Date Contacted: 07/26/2017

If you have animals listed in Pain Category D or E in paragraph VI.A. above, the AWA requires that you formally consult with the IACUC attending veterinarian (AV) or his designee on all aspects of pain and / or distress management. This must be done prior to submission of the proposal to the IACUC / URCO. (Reference IACUC Guideline #22. To facilitate scheduling the AV consultation, please contact Ms. Shirley Whitney in the CMG office (103 Coles Hall, 532-5640, or swhitney@vet.k-state.edu) *Important note: the AV consult is not the IACUC review of your proposal. Please understand that the IACUC committee is autonomous and members will likely ask different questions they deem appropriate during the actual committee review.

E. **Prolonged Restraint:** ☐ **Yes** ☑ **No** (Describe and justify any plans for prolonged restraint >15 min. Reference IACUC Guideline #2)

F. **Pain or Distress Alleviation** - Will you be administering drugs or compounds for sedation, anesthesia or analgesia as a premedication or for anesthetic induction or maintenance? ☐ **Yes** ☑ **No** **(If "YES", all animals receiving the drug or compound will need to be placed in USDA Pain Category D.)**

1. List all drugs or compounds being used for sedation, anesthetic or analgesia during the course of your procedure. Included drug/compound name, dosage, route and frequency.

Drug/Compound	Dosage	Route	Frequency
Dexmedetomidine hydrochloride (Dexdormitor) (Note: The mcg/kg dosage decreases as body weight increases.)	23-25 mcg/kg (0.4-05 ml/dog) or 16.8-19.6 mcg/kg (0.3-0.4 ml/dog)	Intramuscular (IM) or Intravenous (IV)	Once prior to euthanasia to draw the blood via cardiac puncture

2. How will you monitor the animal to ensure the animal is properly anesthetized?

A CMG veterinarian will monitor the vital signs using a stethoscope and clinical assessment to determine and confirm that the animal is properly anesthetized.

G. **Surgery** ☐ **Yes** ☑ **No**

(Reference IACUC guidelines #4, #10)

1. **Procedure** (Describe surgical procedures planned)

2. **Location** (Where is the surgical procedure to be performed?)

3. **Surgeon/Qualifications** (Who will perform procedures? List their training and qualifications.)

4. **Multiple Survival Surgery Procedures** ☐ **Yes** ☑ **No** (If yes, please provide justification)

(Reference IACUC guideline #7)

5. **Non-Survival Surgery Procedures** ☐ **Yes** ☑ **No**

H. **Animal Monitoring -** For protocol purposes, a procedure is defined as an action performed on an animal for research or teaching purposes that has the potential to cause pain or distress to that animal. In order to evaluate pain and/or distress, the KSU IACUC requires an approved plan of how pain or distress will be minimized and documentation of how observations of animals will be recorded.

All procedures performed upon an animal should be listed on an **Animal Monitoring Plan (AMP)** form which is submitted with your IACUC protocol. The AMP form along with the **Animal Observation Record (AOR)** detail how you will observe your animals and what actions you will take in order to minimize pain or distress associated with your research project. Examples of when theses forms would be required include animals that undergo a surgical procedure, animals that undergo anesthesia, animals experimentally infected with an infectious disease, or animals inoculated with potential tumor forming cells. Exceptions to the use of the AMP and AOR would be simple procedures with minimal physiological effect upon the animal, examples of which include vaccination, blood collection, or injection of experimental compounds.

Please complete and submit the AMP with the Protocol application. A link to these forms along with further directions can be found at the KSU IACUC home page. Since the IACUC may follow up on compliance with this requirement, you should maintain these records with your study records after the end of the research project.

If an AMP is included in my approved IACUC document, I understand that it is my responsibility as the PI to assure that the AMP activities will be used as described in the approved protocol. I also understand that should oversight bodies request them, it is my responsibility to be able to document the activities called for in the AMP.

1. Does this protocol require the use of the AMP and AOR? ☑ **Yes** ☐ **No**
(Checking "YES" will make the AMP form appear on the next page)

2. Is an AMP completed? ☑ **Yes** ☐ **No**

3. Indicate where the AMP will be kept (i.e. animal room posted on wall, lab or barn office).

AMP will be posted inside the animal monitoring room for the easy access to the CMG staff and veterinarians. Typically, Dr. Sally Olsen or her associates will oversee the AMP on a regular basis.

Animal Monitoring Plan

Protocol #: | PI: Roman Ganta | PI Contact #: 532-4612 (O); [redacted]

Animal/Group ID: | Species: | Animal Location:

Procedure: | Date of Procedure:

I. Post-Procedure Care (if applicable)

A. List all drugs/medications to be given following the procedure (include name, dose, route, and frequency)

Drug/Medications	Dose	Route	Frequency
Doxycycline	10mg/kg body wt	oral	as per DVM orders

B. List all other care to be provided following the procedure and note frequency.

Post-Procedure Care	Frequency

II. Observations

A. Observation Frequency: daily (twice a day initially)

B. When will the animal be returned to its cage/pen:

C. List the parameters to be monitored, criteria to monitor for and directions for recording, and the appropriate action to be taken if necessary.

Parameter	Monitoring Criteria	Intervention
Temperature	Daily monitoring and in addition when an animal exhibits visible changes in its activity	If an animal has temperature above 106 F, the animal may be on a doxycycline treatment course (if recommended by the attending veterinarian and if this option is preferred over euthanasia).
CBC	Twice weekly for the first two weeks	If an animal is severely anemic (as judged from the CBC analysis where PCV value is less than 35%) or significant change in differential blood counts, we will consult with the assigned CMG veterinarian to initiate appropriate course of action.
Animal Behaviour	depressed, lethargic for more than 24 hrs	CMG veterinarian will be contacted for appropriate action.
Appetite	daily monitoring for the eating pattern and assess changes in the apetite	CMG veterinarian will be contacted for appropriate action.

III. Contact Information:

	Name	Telephone Number
PI	Roman Ganta	785-532-4612 (O)[redacted]
Co-Investigator		
Co-Investigator		
Veterinarian		

In the event that the investigators or the responsible veterinarian cannot be reached or if you have concerns about an animal's care, please contact the KSU Attending Veterinarian (785-532-5648).

I. Animal Manipulations:

1. **List all other drugs and compounds** that you will be administering other than those listed above in Pain or Distress Alleviation (Section F), on the Animal Monitoring Plan (Section H) or in Euthanasia (Section J.8). Include drug, dosage, route and frequency.

Drug/Compound	Dosage	Route	Frequency
Ehrlichia chaffeensis mutants	$1X10^8$ bacteria/ml	I/V	Once for each experiment
Diphenhydramine (Benadryl)	1 mg/lb body weight	oral	Prior to each inoculum

2. **List any rooms where procedures with animals are done (excluding housing and surgery).** Locations for procedures such as behavior testing, treadmill training, blood draws, injections, gavage, etc. should be listed in this chart. If procedures are performed within CMG facilities, the "CMG assigned".

Building/Room Number	Procedure

3. **Biosamples:** ☑ **Yes** ☐ **No** (list type & amount, i.e., phlebotomy, minor biopsies, ascitic fluids, etc.)

Approximately 10 ml blood will be collected twice weekly in EDTA from cephalic veins and or in sodium heparin or red tubes using vaccutainer tubes with 19-22G needles for the first two weeks for all three experiments and once a week there after. The blood will be collected from jugular vein or anterior cephalic or lateral cephalic vein. Postmortem tissue sampling will include the collection of blood (about 50 ml), spleen, liver, lymph nodes, lung, and bone marrow and they will be used for final detailed assessment of infection status. A total of a maximum of 11 blood samples from each animal will be collected.

4. **Tissue Sharing:** ☐ **Yes** ☑ **No** **(**detail any tissue sharing you plan with other investigators)

5. **Other Procedures:** (list any other procedures you might perform on animals in this project)

6. **Adjuvants:** ☐ **Yes** ☑ **No** (explain any adjuvant use. Reference IACUC guideline #12)

7. **Chemical Grade Drugs:** ☐ **Yes** ☑ **No** (If you plan to use a chemical grade please list and provide a scientific explanation for its use; **Reference IACUC guideline # 19)**

J. Veterinary Care:

1. **Animal Housing:** (Provide specific information on where the animals will be housed for your activity.)
PLEASE INCLUDE ROOM NUMBER IF KNOWN

[redacted]

2. **Social/Paired Housing:** (Social animals should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility. "The Guide" 8th Edition):

☑ **Yes** ☐ **No** My animals will be housed in stable pairs or compatible groups?

If no, please provide an adequate justification for an exception to this guidance.

3. **Special Husbandry Considerations:** (Animals will be housed in designated animal rooms/areas, unless approved by the IACUC. Detail special husbandry requirements, i.e. special diets, micro-isolators, etc.):

4. **Animal Surveillance:** (Who observes the animals daily for health problems?)

Our scientific staff and CMG veterinarians

5. **Veterinary Clinical Care:** (Who will you contact if there is a health problem requiring veterinary care?)

CMG staff veterinarians

6. **Wire Bottom Rodent Caging:** If you are using rodents, do you propose to house them in wire-bottom cages?

☐ **Yes** ☐ **No** (If yes, you must explain the rationale for the use of wire bottom cages scientifically. See IACUC Guideline #14)

☑ **N/A**

7. **Study Endpoint** (Experimental studies may involve procedures that cause clinical symptoms or morbidity in animals. The IACUC must consider the selection of the most appropriate endpoint(s). This requires careful consideration of the scientific requirements of the study, expected and possible adverse effects research animals may experience (pain, distress, illness, etc.), the most likely time course and progression of those adverse effects, and the earliest most predictive indicators of present or impending adverse effects. Optimally, studies are terminated when animals begin to exhibit clinical signs of disease if this endpoint is compatible with meeting the research objectives. Such endpoints are preferable to death or moribundity as endpoints since they minimize pain and distress. **The use of death of the animal as an endpoint is strongly discouraged and must be justified to the IACUC - Reference IACUC guideline # 13**. Please describe the endpoint of your study):

After about 60 days of study all animals used in the three experiments will be euthanized .

8. **Euthanasia:** (Reference the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition, link available on the KSU IACUC or the AVMA website, https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx)

Will animals be euthanized as a part of your protocol? ☑ **Yes** ☐ **No**

i. **Method** (include drug, dosage, and route)

Commercial euthanasia drugs like Fatal- plus (Pentobarbital Sodium) at a dose rate of 0.22 ml/kg body wt (390 mg/ml) will be administered.

ii. **Name of person(s) responsible for performing the euthanasia.**

CMG staff veterinarian

9. **Animal Disposition** (what is your plan for the animals after the study is over?)

☑ **Euthanasia** ☐ **Adoption** ☐ **Long-term holding**

☐ **Transfer to another investigator with approved or pending protocol.**

Name:

☐ **Other**

VII. Investigator & Technician Qualifications/Training

(The Animal Welfare Act and the PHS Policy requires that personnel are appropriately trained in animal care and use matters, and that the professional training is documented. The PI is responsible for ensuring that all study personnel have completed appropriate professional training. **Prior to final approval of an animal care and use protocol, the IACUC requires completion of the required activity specific online training for all personnel listed as participating in the animal care and use activity.** The URCO will have access to documentation of completion of the online training through CITI. All other training documentation is the responsibility of the PI. List all persons involved in your activity below - excluding CMG and LACS personnel - and their professional training. Contact the University Research Compliance Office, 532-3224 for information or guidance on animal care and use training)

Name	Training and experience with animals
Roman R. Ganta	PhD; worked with mice, deer and dogs; 15 years
Arathy Nair	DVM, PhD; Experience handling deer (2 years) ,dogs (5 years) , Sheep (3 months)
Huitao Liu	PhD; worked with dogs; 6 years.
Ying Wang	PhD; worked with dogs; 2 years
Deborah Jaworski	PhD; worked with dogs for 5 years; worked with other animal models in excess of 5 years (sheep, deer, cattle, rats, mice, rabbits)
Katheline Trinkl	Undergraduate student; worked with dogs; 1 year
Paidashe Hove	Ph D; worked with large animals for 5 years, he doesn't have experience working with dogs and so he will be trained by our staff or one of the CMG staff members.

The IACUC is required to review and approve changes in personnel for research or teaching involving animals. **Consequently, you must inform the IACUC (via protocol modification) of any changes in animal care research personnel that may occur in your activity. Additionally, you must ensure that new personnel involved in your activity are qualified, have completed the mandatory animal care and use training, and are enrolled in the occupational health and safety program.

☑ **Yes** ☐ **No** **Will personnel be trained in humane handling of this species?**

☑ **Yes** ☐ **No** **Are all personnel enrolled in the KSU Animal Worker Occupational Health and Safety Program?**
(If no, forms can be downloaded from http://www.k-state.edu/research/comply/iacuc/ohsp or you may contact the University Research Compliance Office (2-3224) for information.)

☐ **Yes** ☑ **No** **Will you need animals for protocol-related training purposes, i.e., experimental or surgical technique development or refinement, etc.?** If yes, please specify the technique or procedure to be performed during training (you may reference detailed description in another section of the proposal if appropriate):

Number of animals required to accomplish the proposed training (be sure to include the number of animals requested for training purposes in the total number of animals listed in Section V.D., and Section VI.A.):

Please indicate how training is/will be accomplished:

☑ **Yes** ☐ **No** **Training and/or orientation with P.I., CMG or LACS personnel**

☐ **Yes** ☑ **No** **Instruction by supervising animal caretaker**

☐ **Yes** ☑ **No** **Viewing of instructional videos**

☐ **Yes** ☑ **No** **Other (please specify)**

☑ Yes ☐ No **Individual Technical Procedure Training Form.**

if you marked no, explain below how you are going to document training or technical competence for personnel to perform the procedure(s) proposed.

If you are proposing to use a technical, manipulative, or invasive procedure on animals as part of your activity, it is a requirement that you document the competence of your staff to perform the proposed procedure. Documentation of training is necessary for all personnel for specific animal use procedures such as handling, stomach tubing, euthanasia, injections, biopsy, phlebotomy, restraint, etc. This formal training documentation should be maintained in the laboratory or close by and be readily available for IACUC, USDA, AAALAC, OLAW and research compliance review as appropriate. It is the PI's responsibility to ensure that adequate training is performed, and documented. If you need assistance with training for technical procedures, contact the attending veterinarian (532-5648) or the university veterinarian (532-3224) for advice or assistance.

VIII. Hazardous Material Use: (explain if are you using hazardous materials in your study)

1. ****Biological, Infectious or Parasitic agents** ☐ No ☑ Yes (list)

Ehrlichia chaffeensis and nymphal stage *Amblyomma americanum* ticks

2. ****Recombinant or synthetic nucleic acid molecules** ☑ No ☐ Yes (list)

3. **Hazardous chemicals** ☑ No ☐ Yes (list)

4. **Radioisotopes** ☑ No ☐ Yes (list)

5. **Other** ☑ No ☐ Yes (list)

6. **Select Agents:** Are you using or planning to use agents listed in the Federal Select Agent Program. (http://www.selectagents.gov/SelectAgentsandToxinsList.html)?

☑ No ☐ Yes (list)

The Federal Select Agent Program (www.selectagents.gov), a joint program of the Centers for Disease Control and Prevention (CDC), and the USDA Animal and Plant Health Inspection Service (APHIS), oversees the activities of possession, use and transfer of biological agents and toxins that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products. The program currently requires registration of facilities including government agencies, universities, research institutions, and commercial entities that possess, use or transfer biological agents and toxins.

If you plan to use or are using any of the viruses, bacteria, fungi, rickettsial agents, or toxins on the select agent list, please contact the K-State Responsible Official for select agent use at the Biosecurity Research Institute (785-532-3248), or the URCO (785-532-3224) for information.

(If "yes" you must have a Registration Document from the Institutional Biosafety Committee)**

IBC Registration Document # [redacted] **Approval Date** pending

IX. Extramural Funding: (It is critical that animal care and use procedures detailed in the IACUC protocol are consistent with external funding proposals documents. Discrepancies between the two documents in animal care and use procedures could jeopardize individual and/or institutional funding and compliance. If you make changes, or they are required by the IACUC, it is your responsibility to ensure that grant or funding agencies are informed.)

☑ Yes ☐ No All animal care and use procedures described in this proposal are consistent with those described in external funding applications/documents. If no is checked, please contact the URCO (532-3224).

☐ N/A

X. Clinical Research: (Does this activity involved client owned animals with naturally occurring, or pre-existing conditions?)

☐ Yes ☑ No

XI. **USDA Regulated Activities:** (Is your activity regulated by provisions of the Animal Welfare Act?) Contact the URCO or the attending veterinarian if you need clarification.

Regulated animals would include: - Any live or dead dog, cat, monkey, guinea pig, hamster, rabbit, or warm-blooded animal used for biomedical research, teaching, testing, experimentation, or exhibition purposes. Exemptions to this definition are listed below.

Exempt or non-USDA regulated animals would include: (1) lab rats and mice (*Mus* / *Rattus*) bred for use in research, (2) birds, (3) horses not used for (biomedical) research purposes, and (4) other farm animals such as, livestock or poultry, used or intended for use as food or fiber, or improving animal nutrition, breeding, management, production efficiency, or for improving food or fiber quality.

- ☑ **Yes -** My activity involves species COVERED by the definition of animal in the Animal Welfare Act.
- ☐ **No -** My activity involves animals that are **EXEMPT** from coverage by the USDA
- ☐ **Both** - My activity involves both covered and exempt species.
- ☐ **Also** - My activity involves NIH Regulated Activities (use of any **vertebrate species**).

XII. **Wildlife or Field Investigation:**

☐ **Yes** ☑ **No** Does your activity involve the use or observation of nondomesticated vertebrate species under field conditions?

If "YES," please answer the following:

☐ **Yes** ☐ **No** Does your wildlife field activity require any international, federal, state or local permits?

☐ **Yes** ☐ **No** Are you using any relevant professional society guidelines that are available for your wildlife field activity ?

Online Required Training

TRAINING REQUIREMENTS HAVE RECENTLY CHANGED

The IACUC requires mandatory training prior to protocol approval. Training is now offered through the Collaborative Institutional Training Initiative (CITI) Program. Instructions to register and access training are found on the URCO website: http://www.k-state.edu/research/comply/

Use the check boxes below to select the training courses that apply to this protocol. If you have any questions about training, contact URCO at comply@k-state.edu, or (785) 532-3224.

Mandatory Training

Required for all Principal Investigators, research staff and students

☒ Responsible Conduct of Research ☒ Working with the IACUC

Required (Provost-mandated) for all full time K-State employees

☐ Export Compliance

Species-specific training (check all that apply to this protocol)

☐ Swine ☐ Cattle ☐ Rat ☐ Mouse ☐ Guinea Pig ☐ Hamster ☐ Ferret

☑ Dog ☐ Cat ☐ Horse ☐ Gerbil ☐ Sheep or Goat ☐ Rabbit ☐ Zebrafish

☐ Fish (except zebrafish) ☐ Amphibians ☐ Wildlife (except fish) ☐ Farm Animals or Agricultural Animals

Required procedure-specific training (check all that apply to this protocol)

☐ Survival Surgery

☐ Rat or Mouse, Category D or E procedures

☐ Antibody Production

All new personnel or personnel with expired training are required to register for CITI and take the new training requirements. If you previously completed online IACUC modules, your training status will remain current until it expires. URCO will verify training from the previous system as well as the new system prior to approval of any protocol.

POST APPROVAL MONITORING: The URCO has a Post-Approval Monitoring (PAM) program to help assure that animal care and use activities are performed in accordance with provisions or procedures approved by the IACUC. Accordingly, the URCO staff will arrange PAM visits as appropriate to assess compliance with approved activities.

INVESTIGATOR ASSURANCE FOR THE HUMANE CARE AND USE OF ANIMALS FOR TEACHING AND RESEARCH

(Print this page separately because it requires a signature by the PI.)

P.I. Name: Roman R. Ganta

Title of Project: Vector and host contributions to the regulation of E. chaffeensis gene expression

XIII. ASSURANCES: As the Principal Investigator on this protocol, I provide assurances for the following:

A. **Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, and in accordance with applicable laws, regulations, and guidelines. Any deviation or modification from the procedures detailed herein, must receive prior approval from the Institutional Animal Care and Use Committee (IACUC).**

B. **Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.**

C. **Statistical Assurance: I assure that there has been an adequate evaluation of the experimental design or strategy of this proposal, and that the minimum number of animals needed for scientific validity are used.**

D. **Oversight: All experiments, surgeries, or manipulations involving live animals will be performed under my supervision or that of another qualified individual. In procedures involving USDA Pain Category D or USDA Pain Category E, I have consulted with the attending veterinarian on minimizing pain and/or distress.**

E. **Biohazard\Safety: I assure that in planning this proposal, I have made the proper consideration regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant DNA issues, etc. Additionally, personnel on my study with contact with animals are enrolled in the Animal Worker Occupational Health and Safety Program.**

F. **Training: I assure that personnel performing animal procedures\manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures\manipulations. Inexperienced personnel will be properly trained and/or supervised. Additionally, I understand that I must maintain documentation of appropriate animal care and use training for personnel involved in my study.**

G. **Adverse Event Notification: In compliance with provisions of both the "Ag" and "ILAR Guide," I assure that I will notify the IACUC Attending Veterinarian (Dr. Marlow) if there is a significant unanticipated adverse event during the execution of my activity. This would include unexpectedly high levels of mortality or development of a new disease condition that affects the health and / or welfare of the animals, etc.**

H. **Extramural Funding: If funded by an extramural source, I assure that this application accurately reflects all procedures involving laboratory animal subjects as described in the proposal to the funding agency. (standards are the same, regardless of funding sources).**

I. **Study Duration: I understand that proposals are approved for 3 years. I also understand that as subsequent annual reviews are conducted, it is my responsibility to provide timely and accurate annual review information when requested, to include notification of the IACUC and the University Research Compliance Office (URCO) when my study is completed.**

You may sign this form using a digital signature. DO NOT sign the form until it has been completed.
You cannot edit the form entries once the form has been digitally signed. If you are making revisions to a previously signed form, right-click the digital signature and select Clear to remove the signature (this can only be done by the person who originally digitally signed the form). Forms that have not been signed will not be accepted.

P.I. Signature: Roman R. Ganta Digitally signed by Roman R. Ganta DN: cn=Roman R. Ganta, o=Kansas State University, ou=DMP, CVM, email=rganta@vet.k-state.edu, c=US Date: 2019.02.18 10:11:31 -06'00' Date:

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

RECORD OF DISPOSITION OF DOGS AND CATS

FORM APPROVED OMG NO. 0579-0036
DATE OF DISPOSITION: 02/13/2019

2. PAGE 1 OF 2

[X] SALE [ ] EXCHANGE OR TRANSFER [ ] DONATION

INSTRUCTIONS: COMPLETE APPLICABLE ITEMS 1 THROUGH 8. ORIGINAL AND USDA COPY TO BE RETAINED BY SELLER
BUYER'S COPY TO ACCOMPANY SHIPMENT. IT MUST BE RETAINED BY BUYER

3. SELLER OR DONOR *(NAME & ADDRESS)*
[redacted]

4. BUYER OR RECEIVER *(NAME & ADDRESS)*
KANSAS STATE UNIVERSITY
[redacted]

3A. DEALER'S LICENSE NO. OR RESEARCH FACILITY REGISTRATION NO. (SELLER)
[redacted]

4A. USDA LICENSE NO. OR RESEARCH FACILITY REGISTRATION NO. (IF ANY)

5. IDENTIFICATION OF EACH ANIMAL BEING DELIVERED (SEE REVERSE FOR BREED ABBREVIATIONS FOR DOGS AND CATS) * IF MIXED BREED, LIST 2 DOMINANT BREEDS

COMPLETE ITEMS A THRU G FOR EACH ANIMAL

	IDENTIFICATION NUMBER	DOG "X" M OR F	CAT "X" M OR F	AGE OR DATE OF BIRTH	WEIGHT	BREED OR TYPE	DESCRIPTION OF ANIMAL (COLOR, DISTINCTIVE MARKS, HAIR, TAIL, TATTOOS, ETC.)
8	BCQ-8	M X F	M F	8/12/18	6.40	BEAGLE	TRICOLOR
9	CTQ-8	M X F	M F	8/8/18	7.60	BEAGLE	TRICOLOR
1	DPQ-8	M X F	M F	8/7/18	7.10	BEAGLE	TRICOLOR
5	HO-8	M X F	M F	7/19/18	7.50	BEAGLE	TRICOLOR
16	OIO-8	M X F	M F	7/19/18	6.90	BEAGLE	TRICOLOR
8	QJO-8	M X F	M F	7/19/18	7.10	BEAGLE	TRICOLOR
7	SZO-8	M X F	M F	7/28/18	6.40	BEAGLE	TRICOLOR
6	TDO-8	M X F	M F	7/27/18	7.20	BEAGLE	TRICOLOR
5	TKO-8	M X F	M F	7/29/18	6.60	BEAGLE	TRICOLOR
2	TXO-8	M X F	M F	7/26/18	6.70	BEAGLE	TRICOLOR
14	TZO-8	M X F	M F	7/26/18	6.90	BEAGLE	TRICOLOR
3	UHO-8	M X F	M F	7/28/18	6.00	BEAGLE	TRICOLOR
13	UIO-8	M X F	M F	7/28/18	7.10	BEAGLE	TRICOLOR
4	VDO-8	M X F	M F	7/27/18	7.10	BEAGLE	TRICOLOR

6. DELIVERY BY (CHECK ONE AND COMPLETE APPLICABLE ITEM 7 AND 8)
[ ] COMMERCIAL SHIPPER [ ] BUYER'S VEHICLE [X] SELLER'S VEHICLE

7. NAME AND ADDRESS OF COMPANY OR FIRM (INCLUDE ZIP CODE)
[redacted]

8. NAME AND BUSINESS ADDRESS OF TRUCK DRIVER (INCLUDE ZIP CODE)
[redacted]

9. RECEIVED BY	10. SIGNATURE	11. TITLE	12. DATE

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

RECORD OF DISPOSITION OF DOGS AND CATS

[X] SALE [ ] EXCHANGE OR TRANSFER [ ] DONATION

FORM APPROVED OMG NO. 0579-0036
DATE OF DISPOSITION
02/13/2019

2. PAGE
2 OF 2

DEALER'S LICENSE NO. OR RESEARCH FACILITY REGISTRATION
[redacted]

3. SELLER OR DONOR *(NAME & ADDRESS)*
[redacted]

4. BUYER OR RECEIVER *(NAME & ADDRESS)*
KANSAS STATE UNIVERSITY
[redacted]

IDENTIFICATION NUMBER	DOG "X" M OR F	CAT "X" M OR F	AGE OR DATE OF BIRTH	WEIGHT	BREED OR TYPE	DESCRIPTION OF ANIMAL (COLOR, DISTINCTIVE MARKS, HAIR, TAIL, TATTOOS, ETC.)
10 · WYQ-8	M X F	M F	8/5/18	7.20	BEAGLE	TRICOLOR
12 · XEO-8	M X F	M F	7/31/18	6.00	BEAGLE	TRICOLOR
12 · YZQ-8	M X F	M F	8/4/18	6.80	BEAGLE	BLOND
10 · ZTO-8	M X F	M F	7/31/18	7.10	BEAGLE	TRICOLOR
	M F	M F				
	M F	M F				
	M F	M F				
	M F	M F				
	M F	M F				
	M F	M F				
	M F	M F				
	M F	M F				
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	M F	M F				
	M F	M F				
	M F	M F				
	M F	M F				
	M F	M F				

This record is required by law (7 USC 2131-2156). (9 CFR, Subchapter A, Parts 1, 2 and 3). Failure to maintain this record can result in a suspension or revocation of license and/or imprisonment for not more than 1 year, or a fine of not more than $1,000, or both.

RECORD OF ACQUISITION AND DOGS AND CATS ON HAND

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

See reverse side for OMB information

FORM APPROVED
OMB NO. 0579-0036

1. RECORD FOR ("X")
☐ Dealer ☐ Holding Facility (Submit copy to Dealer)
☒ Other ☐ Exhibitor (Dogs and Cats only)

USDA LICENSE OR REGISTRATION NO. [redacted]

2. NAME AND ADDRESS OF LICENSEE, REGISTRANT, OR HOLDING FACILITY
Kansas State University, VP Research, Comparative Medicine Group [redacted]

3. BUSINESS YEAR
FROM (Mo, Day, Yr.) 10/01/18
TO (Mo., Day, Yr.) 09/30/19

4. PAGE NO. 03

IDENTIFICATION OF EACH ANIMAL BEING DELIVERED (See reverse for Breed Abbreviations)

ACQUIRED FROM

DISPOSITION

A. TATTOO OR USDA TAG NO.	B. DOG "X" M or F	C. CAT "X" M or F	D. AGE OR DATE OF BIRTH	E. WT.	F. BREED OR TYPE (If mixed breed, list 2 dominant breeds)	G. DESCRIPTION OF ANIMAL (Color, Distinctive Marks, Hair, Tail Tattoos, etc.)	H. DATE ACQUIRED	I. NAME AND ADDRESS USDA LICENSE OR REGISTRATION NUMBER, OR DRIVER'S LICENSE NUMBER AND STATE, VEHICLE LICENSE NUMBER AND STATE,	J. Date Removed or Sold	K. Date Died or Euthanized (Specify)
13-19C	M X F	M F	07/28/18	7.10	Beagle	Tri-Color Tattoo: UIO8	02/14/19	[redacted]		Euth. 4/24/19
14-19C	M X F	M F	07/26/18	6.90	Beagle	Tri-Color Tattoo: TZO8	02/14/19			Euth. 4/24/19
15-19C	M X F	M F	07/29/18	6.60	Beagle	Tri-Color Tattoo: TKO8	02/14/19			Euth. 4/24/19
16-19C	M X F	M F	07/19/18	6.90	Beagle	Tri-Color Tattoo: OIO8	02/14/19			Euth. 4/24/19
17-19C	M X F	M F	08/04/18	6.80	Beagle	Blonde Tattoo: YZQ8	02/14/19			Euth. 4/24/19
18-19C	M X F	M F	07/19/18	7.10	Beagle	Tri-Color Tattoo: QJO8	02/14/19			Euth. 4/24/19

APHIS FORM 7005 (JUN 95)	INSPECTOR USE ONLY	LAST INSPECTION (Date)	TOTAL NO. ANIMALS ENTERED SINCE LAST INSPECTION	COUNT TOTAL NO. ANIMALS ACTUALLY ON PREMISES	DIFFERENCE (+ OR -)	DATE	INITIALS

(Replaces VS Form 18-5 which may be used.)

This record is required by law (7 USC 2131-2156). (9 CFR, Subchapter A, Parts 1, 2 and 3). Failure to maintain this record can result in a suspension or revocation of license and/or imprisonment for not more than 1 year, or a fine of not more than $1,000, or both.

RECORD OF ACQUISITION AND DOGS AND CATS ON HAND

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

See reverse side for OMB information

FORM APPROVED
OMB NO. 0579-0036

1. RECORD FOR ("X")

☐ Dealer ☐ Holding Facility (Submit copy to Dealer)
☒ Other ☐ Exhibitor (Dogs and Cats only)

USDA LICENSE OR REGISTRATION NO. [redacted]

2. NAME AND ADDRESS OF LICENSEE, REGISTRANT, OR HOLDING FACILITY

Kansas State University, VP Research, Comparative Medicine Group [redacted]

3. BUSINESS YEAR

FROM (Mo, Day, Yr.) 10/01/18
TO (Mo., Day, Yr.) 09/30/19

4. PAGE NO. 02

IDENTIFICATION OF EACH ANIMAL BEING DELIVERED (See reverse for Breed Abbreviations)

ACQUIRED FROM

DISPOSITION

A. TATTOO OR USDA TAG NO.	B. DOG "X" M or F	C. CAT "X" M or F	D. AGE OR DATE OF BIRTH	E. WT.	F. BREED OR TYPE (If mixed breed, list 2 dominant breeds)	G. DESCRIPTION OF ANIMAL (Color, Distinctive Marks, Hair, Tail Tattoos, etc.)	H. DATE ACQUIRED	I. NAME AND ADDRESS USDA LICENSE OR REGISTRATION NUMBER, OR DRIVER'S LICENSE NUMBER AND STATE, VEHICLE LICENSE NUMBER AND STATE,	J. Date Removed or Sold	K. Date Died or Euthanized (Specify)
07-19C	M X F	M F	07/28/18	6.40	Beagle	Tri-Color Tattoo: SZO8	02/14/19	[redacted]		Euth. 4/23/19
08-19C	M X F	M F	08/12/18	6.40	Beagle	Tri-Color Tattoo: BCQ8	02/14/19			Euth. 4/23/19
09-19C	M X F	M F	08/08/18	7.60	Beagle	Tri-Color Tattoo: CTQ8	02/14/19			Euth. 4/23/19
10-19C	M X F	M F	08/05/18	7.20	Beagle	Tri-Color Tattoo: WYQ8	02/14/19			Euth. 4/24/19
11-19C	M X F	M F	07/31/18	7.10	Beagle	Tri-Color Tattoo: ZTO8	02/14/19			Euth. 4/24/19
12-19C	M X F	M F	07/31/18	6.00	Beagle	Tri-Color Tattoo: XEO8	02/14/19			Euth. 4/24/19

APHIS FORM 7005 (JUN 95)	INSPECTOR USE ONLY	LAST INSPECTION (Date)	TOTAL NO. ANIMALS ENTERED SINCE LAST INSPECTION	COUNT TOTAL NO. ANIMALS ACTUALLY ON PREMISES	DIFFERENCE (+ OR -)	DATE	INITIALS

(Replaces VS Form 18-5 which may be used.)

This record is required by law (7 USC 2131-2156). (9 CFR, Subchapter A, Parts 1, 2 and 3). Failure to maintain this record can result in a suspension or revocation of license and/or imprisonment for not more than 1 year, or a fine of not more than $1,000, or both.

RECORD OF ACQUISITION AND DOGS AND CATS ON HAND

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

See reverse side for OMB information

FORM APPROVED
OMB NO. 0579-0036

1. RECORD FOR ("X")
☐ Dealer ☐ Holding Facility (Submit copy to Dealer)
☒ Other ☐ Exhibitor (Dogs and Cats only)

USDA LICENSE OR REGISTRATION NO. [redacted]

2. NAME AND ADDRESS OF LICENSEE, REGISTRANT, OR HOLDING FACILITY
Kansas State University, VP Research, Comparative Medicine Group
[redacted]

3. BUSINESS YEAR
FROM (Mo, Day, Yr.): 10/01/18
TO (Mo., Day, Yr.): 09/30/19

4. PAGE NO. 01

IDENTIFICATION OF EACH ANIMAL BEING DELIVERED (See reverse for Breed Abbreviations)

ACQUIRED FROM

DISPOSITION

A. TATTOO OR USDA TAG NO.	B. DOG "X" M or F	C. CAT "X" M or F	D. AGE OR DATE OF BIRTH	E. WT.	F. BREED OR TYPE (If mixed breed, list 2 dominant breeds)	G. DESCRIPTION OF ANIMAL (Color, Distinctive Marks, Hair, Tail Tattoos, etc.)	H. DATE ACQUIRED	I. NAME AND ADDRESS USDA LICENSE OR REGISTRATION NUMBER, OR DRIVER'S LICENSE NUMBER AND STATE, VEHICLE LICENSE NUMBER AND STATE,	J. Date Removed or Sold	K. Date Died or Euthanized (Specify)
01-19C	M X F	M F	08/07/18	7.10	Beagle	Tri-Color Tattoo: DPQ8	02/14/19	[redacted]		Euth 4/23/19
02-19C	M X F	M F	07/26/18	6.70	Beagle	Tri-Color Tattoo: TXO8	02/14/19			Euth. 4/23/19
03-19C	M X F	M F	07/28/18	6.00	Beagle	Tri-Color Tattoo: UHO8	02/14/19			Euth. 4/23/19
04-19C	M X F	M F	07/27/18	7.10	Beagle	Tri-Color Tattoo: VDO8	02/14/19			Euth. 4/23/19
05-19C	M X F	M F	07/19/18	7.50	Beagle	Tri-Color Tattoo: OHO8	02/14/19			Euth. 4/23/19
06-19C	M X F	M F	07/27/18	7.20	Beagle	Tri-Color Tattoo: TDO8	02/14/19			Euth. 4/23/19

APHIS FORM 7005 (JUN 95)	INSPECTOR USE ONLY	LAST INSPECTION (Date)	TOTAL NO. ANIMALS ENTERED SINCE LAST INSPECTION	COUNT TOTAL NO. ANIMALS ACTUALLY ON PREMISES	DIFFERENCE (+ OR -)	DATE	INITIALS

(Replaces VS Form 18-5 which may be used.)

#[redacted] **R. Ganta**, “Vector and host contributions to Ehrlichia chaffeensis gene expression ([redacted])” (Cat D). Dr. Ganta and Dr. Nair were present, via zoom, to provide an overview. Denver Marlow moved and Gayle Willard seconded to require the following modifications to secure approval. Motion passed.

1. On the front page, please check the Research category.
2. On the front page, please provide your email address.
3. Section III.B.2. Please add (1920-present).
4. Section V.A.
    a. Experiment 1: Please change “Tick cells” to read “Tick cells (containers that hold the ticks).”
    b. Experiment 3:
        i. For the Benadryl please change “(1 mg/ml)” to “(1 mg/lb).”
        ii. For the dexdormitor please change “16.8-19.6 mg/kg” to “16.8-19.6 mcg/kg.”
5. Section VI.B.2.2. Please add (1920-current).
6. Section VI.B.3.Replacement: Please add the following keywords: digital, in vitro, computer, virtual, tissue culture. Then please re-do the search and update the narrative as necessary.
7. Section VI.B.3.Refinement: Please update the last part of the sentence to read “through monitoring of clinical signs in accordance with the animal monitoring plan (AMP).”
8. Section VI.B.4.Refinement: Please reword to read “Refinements will be used per AV consult with Dr. Marlow.”
9. Section VI.F.1. Please correct the “05” to “0.5” in the dosage column.
10. Animal Monitoring Plan (AMP)
    a. II.B. Please complete. Will the animals be returned to their pen immediately?
    b. II.C. Please change the temperature 106 to 104.
    c. III. Veterinarian telephone number: Please insert Dr. Sally Olson’s work cell phone 785-410-7513.
11. Section VIII. Once your renewal IBC application is approved, please submit a minor modification to update the IBC number and approval date.

Denver Marlow moved and Gayle Willard seconded to allow review of those modifications by the chair. Motion passed.