FORM A-100

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Colorado State University
Animal Care and Use Research/Teaching Protocol Review Form

IACUC approval of this protocol review form is necessary prior to animals being obtained, housed or manipulated for research or teaching purposes. IACUC approval of activities conducted on animals outside of CSU prior to their arrival to CSU is also necessary.

This form should be used for new protocols, and for renewing protocols at the end of every third approval period. **Submit one signed original and 15 copies** (double-sided preferred) to the Research Integrity and Compliance Review Office, 321 General Services Building. Please make sure that all required signatures are obtained on the final sheet of the form before submission. Answer each question, do not leave questions blank. If the question does not apply to your research, respond with an 'N/A'. Do not answer a question by referring to your response to another question; this form is designed to collect necessary information in a grouped format.

PART I—Basic Protocol Information

1. Investigator Information

a) Principal Investigator: N. Thompson Hobbs
PI must be faculty member, administrative professional, or permanent research associate

b) Department: NREL 4 -Digit Campus Zip Code: 1499

c) Campus Phone: 491-5738

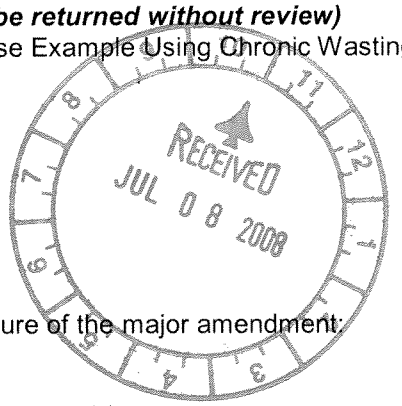
d) E-mail: nthobbs@nrel.colostate.edu

e) Secondary Contact name/phone/email: Dr. Michael Miller, 970-491-1101, mike.miller@state.co.us

f) List researchers and staff qualified to carry out this protocol. If staff listed is involved with *any* **Protocol Procedures,** please fill out a **Training Record (at the end of this form)** for each staff member.
Dr. Michael Miller, Dr. N. Thompson Hobbs

2. Project Information ***(This information must be filled out or your protocol will be returned without review)***

a) Project/ Course Title: Bayesian Hierarchical Modeling of Disease Dynamics - A Case Example Using Chronic Wasting Disease



b) This project is for:
- ☒ Research
- ☐ Teaching

c) This project is a (*check only one*)
- ☒ New project
- ☐ A major amendment to an existing project: IACUC protocol #
  If this is a major amendment, please provide a brief description of nature of the major amendment:
- ☐ A renewal/non-competing or competing continuation: IACUC protocol #

d) Animal acquisition and/or use for this project will be funded through (*check all that apply*):
- ☐ A teaching (1-3 or 1-4) account: Provide fund # if known:
- ☐ An internal funding mechanism: Provide fund # if known:
- ☒ External funding source *(if this is checked you must answer e, below)*

e) If this protocol is for a research project with external funding please answer the following questions, *and you must also include with the A-100 submittal a copy of the face page and sections of the grant proposal that describe animal activities (i.e., materials and methods and vertebrate animals sections, scope of work; etc.)*

Pass number (preferred) or grant fund # 78684
Funding agency: NSF
Status of funding:
- ☐ Funded, funding start date was
- ☒ Notified of pending funding, anticipated award date is 5/15/08
- ☐ Submitted, submission date was
- ☐ In preparation or pending, planned submission date is

☐ Other (explain):

3. Animal Information

Enter one **SPECIES** in each box and report vertically ☐ (if more than 4, list on separate attachment)	**Mule deer (free-ranging)**			
Sex(s):	**either**			
Age/weight range:	**adult**			
NUMBER to be used in Year 1:	**240**			
NUMBER to be used in Year 2:	**180**	Note that most of these 180 animals will be recaptures of animals captured and marked in year 1.		
NUMBER to be used in Year 3:	**180**	Note that most of these 180 animals will be recaptures of animals captured and marked in year 1 and 2.		
TOTAL NUMBER for the lesser of 3 years or duration of project				
SOURCE of animals: NOTE: If this is a study using Client/Student Owned animals, you must provide a copy of the Informed Owner Consent Form along with approval from VMC Director (may be signed on the submitted consent form or provide a memo or email from the VMC Director)	**Northeastern Colorado**			
USDA PAIN CATEGORIES: A painful procedure is defined as any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in an animal to which that procedure is applied. Animals exhibiting signs of pain, discomfort, or distress such as decreased appetite/activity level, decreased mobility, adverse reactions to physical contact, open sores/necrotic skin lesions, abscesses, lameness, conjunctivitis, corneal edema, and photophobia are expected to receive appropriate relief unless written scientific justification is provided in the A-100 protocol and approved by the IACUC. **Indicate which level(s) apply for each species. If listing more than one, indicate how many animals at each pain level. Example: B (20 mice) and C (15 mice). If an animal is used for multiple procedures, count it in the most painful category (see below).** **Category B:** breeding, conditioning only, or holding colony. **Category C:** No more than momentary or slight pain or distress and no use of pain-relieving drugs; or no pain or distress. Examples: euthanized for tissues; observation under normal conditions; positive rewards; routine injections (not Freund's Adjuvant); tattooing, blood sampling. **Category D:** Pain or distress appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress. Examples: Needle biopsy, non-survival or survival surgeries, terminal cardiac blood collection; exposure of blood vessels for catheter implantation; induced infections or antibody production. **Category E:** Unrelieved pain or distress. Examples: toxicological or microbial testing or infectious disease research that requires continuation	**D**			

until clinical symptoms are evident or death occurs; application of noxious stimuli; prolonged restraint; use of paralyzing drugs for restraint; infliction of burns or trauma.				

For any protocols with Pain Category D or E, the USDA requires that PIs consult with the University Veterinarian or designee PRIOR to protocol submission:

Signature of University Vet or designee_____ **Date of consultation** _____
Or you may provide an email to the IACUC coordinator from the University Vet or designee indicating that the consultation has occurred. The UV is Dr. James Owiny 491-5668

3. Animal Information

b) If using animals from other protocols, identify the PI and protocol number, and briefly list the procedures the animals have undergone on the protocols; identify the individual animals by identification number if applicable: n/a

4. Project Activity Duration

a) Start Date *(date you first expect to order/obtain animals)*: 5/15/10
(*Unless continuation,* ***start date should not be prior to IACUC review date***)

b) Stop Date *(date you expect to be finished using animals)*: 5/14/12

5. Project Summary

Provide a summary of the project in <200 words. The lay summary should be readily understandable to the general public and is for example, what would be released to a newspaper if requested. It must explain the need for the research, what the project goals are, and how the use of animals will help reach the stated goals. Please describe why this study is important.

For 4th, 7th, 10th, etc. yr renewals, please provide a summary of what was done during the previous protocol approval period (you can include a list of publications, seminars presented, etc):

During the last decade, chronic wasting disease (CWD) emerged as a disease of potentially enormous significance to the ecosystems and human economies of North America. A fundamental limitation on understanding CWD, as with virtually all emerging infectious diseases, is the inability to forecast behavior of infected populations based on models linked with data from the field and laboratory. For example, earlier work by our research team identified genetic variation that appears to offer resistance to CWD infection. Although this variation could be critical to controlling the disease, understanding its significance requires the ability to model the dynamics of the disease in infected populations, which currently is not feasible. Our work proposed has four aims:

Aim 1: Provide a case example of a novel, general approach for assimilating data with models of emerging infectious diseases.

Aim 2: Evaluate support in data for competing models of transmission of CWD and estimate the average number of new infections contributed by a single infected individual.

Aim 3: Reveal demographic, genetic, and environmental sources of heterogeneity in disease transmission.

Aim 4: Use the models developed under Aim 1 and 2 to evaluate the consequences of CWD for the growth and decline of mule deer populations and the opportunities for controlling CWD.

These aims will be addressed with a field study that provides data for estimating parameters of a population model. The parameterized model will provide a basis for mathematical analysis of long-term dynamics and short-term transient behavior of populations infected with CWD.

6. To help IACUC streamline the review process, please answer the following about procedures involved in your protocol:

a) Deep anesthesia followed by euthanasia of animals and tissue harvest. No other procedures are conducted on animals ..... ☐ Yes ☒ No
b) Deep anesthesia followed by terminal surgery for research or teaching purposes. No other procedures are conducted on animals ..... ☐ Yes ☒ No

c) Only minor procedures with minimal pain or discomfort of the animals (such as blood sampling) Yes (darting wild animals to put collars on; rectal mucosa biopsy)
d) Only observation of field animals ☒ Yes ☐ No (after collars are
e) Only clinical evaluation of animals during routine reevaluations in hospital ☐ Yes ☒ No
f) Involves the use of food animals on studies designed to improve production efficiency and do not involve surgery or other invasive procedures ☐ Yes ☒ No
g) Is this IACUC application solely for a breeding colony at CSU ☐ Yes ☒ No

7. Animal care (if not applicable, mark N/A):

a) Location of housing: NA

b) Location of procedures: Field sites in northeastern Colorado

c) Will Lab Animal Resources provide the daily care ☐ Yes ☒ No
NA

Signature of University Vet or designee_____ **Date** _____
Or you may provide an email to the IACUC coordinator from the University Vet or designee with his/her approval.
The UV is Dr. James Owiny 491-5668

d) What veterinarian will provide medical care to animals?:
☐ LAR or
☒ Other
Specify who: Dr. M. Miller (CDOW) or designee
Contact information: 970-472-4348
If other, justify why LAR will not be providing medical care: Veterinary care is under purview of CDOW.
If other, this must be approved by the University Veterinarian or designee:

Signature of University Vet or designee _____ **Date**_____
Or you may provide an email to the IACUC coordinator from the University Vet or designee with his/her approval.
The UV is Dr. James Owiny 491-5668

e) Location of medical records: NA
f) Are there any adverse effects, (ex.is the lifespan of the animals that you plan to use shortened or are there any health concerns) due to the genotype of the animals?
☐ Yes ☒ No
If yes, please explain: Some animals in the wild *may* have genotypes that convey resistance to CWD. It is the one of the purposes of our work to determine if these genotypes are protective.

g) Exercise/Enrichment NA

8. Living animals are required for this project because:

a) Complexity of the processes studied cannot be duplicated/modeled using in vitro models ☐ Yes ☒ No
b) Not enough information known about processes being studied to design non-living models ☐ Yes ☒ No
c) Pre-clinical studies in living animals are necessary prior to human testing... ☐ Yes ☒ No
d) This study requires tissue harvested from animals prior to in vitro testing.... ☐ Yes ☒ No
e) Currently this is the best method to accomplish the required teaching objectives ☐ Yes ☒ No
f) Populations are being studied in natural or semi-natural environments ☒ Yes ☐ No
g) Animal behavior is being studied ☒ Yes ☐ No
h) Other (please specify):

9. To comply with USDA Policy 12, provide documentation of a literature search to certify that 1) alternatives to each potentially painful/distressful procedure contained in this protocol have been sought, 2) the work is not duplicative of previous studies and 3) the fewest number of animals will be used to obtain valid results.

a) For automated literature searches, provide answers to each question below:
Date search performed: June 2, 2008
Keywords used: mule deer, Chronic Wasting Disease, transmission rate, population model

Period covered by search: 1980-2008
Names of databases searched: Web of Science
Did the search reveal applicable alternatives ..... ☐ Yes ☒ No
If yes, please explain:

b) The Animal Welfare Act allows other means of conducting a search to certify the above. If you used an alternative search strategy, provide information on the strategy, methods and sources: n/a
c) If this is a teaching protocol, please specify why there are no alternatives to using live animals: n/a

10. This species has been selected because:

a) Anatomy, physiology, behavior or agent susceptibility of species uniquely suited to the study	☒ Yes	☐ No
b) Lowest phylogenetic species providing adequate size, tissue, or anatomy for proposed study	☐ Yes	☒ No
c) This species provides a particularly good model for the human or other animal disease or process	☐ Yes	☐ No
d) Previous studies which form the background for this project used this species	☒ Yes	☐ No
e) The objective of this study is to provide information about the target species	☐ Yes	☒ No
f) Other (please specify):		

11. The IACUC requires a power calculation be provided or an explanation why a power calculation is not feasible for this project. Complete one or more of the following to justify the number of animals you will use (you may refer to http://www.math.uiowa.edu/~rlenth/Power/ for help). For experimental designs with multiple groups/treatments, it is suggested that a table of animal numbers per group be provided. In addition make sure the animal numbers justified in # 11 agree with those mentioned in other sections of the A-100 (e.g. # 15 and # 23).

The following was prepared Professor Jennifer Hoeting, Statistics Department, Colorado State University. She is one of the co-principal investigators on the proposed project.

We will use Bayesian hierarchical methods to assimilate population models with field data. Although power analyses are not typically considered under the Bayesian paradigm, an examination of power in the frequentist paradigm may be useful. By adopting a simple model that assumes independent observations over time and space, only one observation of CWD status per deer instead of the multiple observations planned for in the proposal, and one covariate, we can reasonably undertake a power analysis. Using methodology developed by Demidenko (2007), we examined power under a several reasonable scenarios and found high power to detect significant covariate effects for the proposed study. For example, under a simplistic one-covariate logistic regression model and based on models of CWD prevalence for our study area reported in Miller and Conner (Figure 2, 2005), we found the following. Assuming a sample of 300 deer (the total number of animals marked in our study) with probability that the deer is a male equal to 0.3 and probability of infected given male equal to 0.12, the power to detect an odds ratio of 3.2 due to sex with a significance level of 0.05 is 0.90. Thus, any smaller sample size would reduce our power to unacceptable levels for this response. Considering the relationship between genotype and CWD prevalence, we assume that the probability of deer in Colorado are SF equals 0.2 (Jewell et al. 2005, M.K. Watry, 2007, unpublished MS thesis, CSU) and that probability of infected given SF equals 0.004 (Jewell et al. 2005). It follows that the power to detect an odds ratio of 30 of infection (SS /SF , Jewell et al., 2005) with a significance level of 0.05 is 100%. Even if we observe the lower limit of the CI for the odds-ratio (= 4, Jewell et al. 2005) we still have power of 0.99.

Demidenko, E. 2007. Sample size determination for logistic regression revisited. Statistics in Medicine 26:3385-3397.

Jewell, J. E., M. M. Conner, L. L. Wolfe, M. W. Miller, and E. S. Williams. 2005. Low frequency of prp genotype 225sf among free-ranging mule deer (odocoileus hemionus) with chronic wasting disease. Journal of General Virology 86:2127-2134.

Miller, M. W., and M. M. Conner. 2005. Epidemiology of chronic wasting disease in free-ranging mule deer: Spatial, temporal, and demographic influences on observed prevalence patterns. Journal of Wildlife Diseases **41**:275-290.

c) This is a teaching protocol (specify species, number of animals and number of students so that the IACUC can understand the relationships): n/a

d) This study involves tissue or cells harvested from animals for *in vitro* studies (explain the number of animals requested for the amount of tissue needed to obtain a specified level of precision desired, or if an experiment involving the tissue samples will be conducted as part of this protocol, provide power calculations as described in *b* above): n/a

e) This study involves breeding animals (list number of breeding adults used/number of offspring produced each year and describe how the animals are expected to be allocated to the subsequent experiment. Note these experiments will need to submit separate A-100s): n/a

f) This is a study of feral or wild animals where animals will be captured and released attempting to maximize sample size within logistical constraints: ☒ Yes ☐No

g) This is an observational, non-manipulative study in that animal numbers will not be captured or their behavior will not be interfered with and animal numbers cannot be predicted: ☐ Yes ☒No

h) Sample size are government driven or agency mandated: ☐ Yes ☒No
If yes, please provide appropriate references which justifies their requirements:

i) Other (please describe in detail):

12. Is this a field study? ..... ☒ Yes ☐No
If no, move to question 13. If yes, please provide the following information:
a) Briefly describe the capture device (e.g. trap, net, electroshock, etc).

Overview: Mule deer will be captures by using helicopter netgunning contracted to a private vendor. This is a standard deer capture technique previously approved by CDOW ACUC. Once a group of mule deer are located and an animal is randomly selected from the group, it is pursued (typically <1 min) until the netgunner can fire a net over the deer. Male deer in velvet will not be netted. Once the deer becomes entangled in the net, it is restrained, blindfolded, hobbled, and untangled from the net; typically this process takes <5 minutes. The deer is then ferried to a nearby site for processing. As needed to facilitate sampling or extended handling, deer will be sedated using combination of Telazol® (tiletamine HCl and zolazepam; 4.4 mg/kg) and xylazine (2.2 mg/kg), ketamine HCl (2.0 mg/kg) and medetomidine HCl (0.1-0.2 mg/kg), carfentanil citrate (0.03 mg/kg) and xylazine HCl (0.5-1.0 mg/kg), or thiafentanil oxalate (0.1 mg/kg) and xylazine HCl (0.5-1.0 mg/kg) delivered intramuscularly (IM) via syringe. The choice of drugs will be at the discretion of the field veterinarian. Where appropriate, antagonists will be used to reverse anesthetic effects after handling and sampling; for xylazine, yohimbine HCl [0.25 mg/kg intravenously (IV)] or tolazoline [2 mg/kg IV or subcutaneously (SC)]; for medetomidine, tolazoline (2 mg/kg IV or SC) and antipamezole (0.2–0.35 mg/kg IV, or divided IV and SC); for carfentanil, naltrexone HCl (100 mg/mg, divided IV and SC); for thiafentanil, naltrexone HCl (100–150 mg total, IV or SC). Based on previous experience and published data, we anticipate 3–10 min induction times followed by anesthesia or sedation of sufficient duration to allow biopsy, blood collection, marking, and collection of field data, which may take 10–30 minutes.

Command Post: Netgunning will be done at specified locations. Only personnel from the helicopter netgunning service will be involved in the actual capture of mule deer. Personnel from the project (CDOW or CSU) will staff the nearby staging areas during the entire operation, and will be responsible for sampling and marking. Staging areas will change to remain close to the netgunning operation. Two-way radio contact between the staging area and helicopter crew will be maintained throughout the capture process in case problems need to be resolved. The field veterinarian, in consultation with the attending veterinarian as needed, will have ultimate responsibility for decisions that have to be made concerning animal welfare.

Capture Conditions: Mule deer will be captured via netgunning in fall and early winter. Mule deer should be in good condition and temperatures should be cool. Capture operations will be halted if ambient temperature falls below 0°F or exceeds 75°F for ≥0.5 hour. Based on previous experience we anticipate 2% capture related mortality.

Pursuit Time: Individual mule deer will not be pursued for over 3 minutes and chase time will be recorded by a helicopter crew member for each animal. Chases will be aborted if excessive exertion (open-mouthed breathing, stumbling) is noticed or if a deer appears headed for a potentially dangerous situation (fence, road, etc.).

No-Fly Zones: Deer captures will take place on public land in Northeastern Colorado or private land where approved by the landowner. Captures will take place away from roads and man-made structures.

b) What is the maximum amount of time animals will spend in trap or net? < 5 minutes
c) Is there a possibility lactating females will be captured? No.
d) How will animals spending time in capture device be shielded from harsh environmental conditions (e.g. heat, cold, rain, etc.)? Mule deer will be captured via netgunning in fall and early winter. Mule deer should be in good body condition and temperatures should be cool. Capture operations will be halted if ambient temperature falls below 0°F or exceeds 75°F for ≥0.5 hour. Based on previous experience we anticipate 2% capture related mortality.
e) During what season and at what time of day will capture take place? Fall (Sept-Oct)
f) What method of marking animals will be used? In general, toe clipping is not acceptable. Deer will be collared with neckband and VHF or GPS telemetry collars.

g) What insulative bedding will be used? n/a

PART II—Surgical and Other Manipulations

13. Will surgical procedures be involved (Y/N)? ☐ Yes ☒ No
If no, move to question #19. If yes, complete question 13-18, below.
Will any individual animal undergo more than one operative procedure ☐ Yes ☐ No
If yes, please justify:

14. Surgery will be:

a) ☐ Survival OR ☐ Terminal

b) ☐ Major OR ☐ Minor
Major surgery penetrates or exposes a body cavity or produces substantial impairment of physiological or psychological function (e.g. laparatomy, thoractomy, joint replacement, limb amputation).

15. Briefly describe operative procedure or provide IACUC approval number for SOP.

16. Pre-anesthetics, anesthetics, and/or sedatives
(Please provide the following information for each drug used. Complete question 22 below to provide information on analgesia.)

a) Drug:
b) Initial Dose (mg/kg):
c) Route:
d) Supplemental Dose (mg/kg):
Route:
Frequency:

17. Sterile Technique
a) Will sterile instruments be used ☐ Yes ☐ No
If yes, explain method of sterilization:

b) If multiple surgeries will be performed on the same day, how are instruments sterilized between uses?

c) Sterile gloves worn ..... ☐ Yes ☐No
d) Sterile drapes used ..... ☐ Yes ☐No
e) Animal hair/fur/wool clipped ..... ☐ Yes ☐No
f) Explain skin preparation (agent and prep):
g) Sterile gown worn ..... ☐ Yes ☐No
h) Sterile mask worn ..... ☐ Yes ☐No
i) Head cover and foot cover worn ..... ☐ Yes ☐No

Will prophylactic antibiotics be used? ☐ Yes ☐No
If yes, answer the following questions:

What antibiotic will be used?
Dose of antibiotic:
Route of administration:
Timing/duration of administration:
Justification for use of prophylactic antibiotics:

18. Describe anesthetic monitoring and post-operative recovery/care, including frequency and location of post-op monitoring:

19. Non-surgical manipulations (list the following information for each):

Prion infection status will be determined by examining biopsies of rectal mucosa using established methods (González et al. 2005, Spraker et al. 2006, Wolfe et al. 2007). To relieve pain at the biopsy site, a topical analgesic cream (Lidocaine and Prilocaine 2.5%, Fougara® Cream, E. Fougara and Company, Altanta Inc., Melville, NY) will be applied to the distal ~2 cm of rectal mucosa about 5 min before sampling. About 10 min after applying the local anesthetic cream, the rectal mucosal border will be exposed by manually exteriorizing the anal mucosa and isolating it using a disposable speculum (RecSpec™, Veterinary Instrumentation, Jorgensen Laboratories, Loveland, CO). Using Brown-Adson forceps, the mucosa will be lifted from a depression between the rectal columns about 0.8–1 cm rostral to the transition between the anal orifice and the mucosa (muco-cutaneous junction). A small piece (5-6 mm diameter) of the elevated mucosa will be cut with fine point scissors or rectal biopsy forceps. Bleeding will be controlled with direct pressure and Gel Foam® (Pharmacia & Upjohn Company, Pharmacia Corp, Kalamazoo, MI) applied as needed. Additional topical analgesic cream will be applied directly to the biopsy sites, and deer will be given a single prophylactic antibiotic injection. Gloves and disposable biopsy instruments will be used to minimize potential for cross-contamination between animals.

González, L., Jeffrey, M., Sisó, S., Martin, S., Bellworthy, S. J., Stack, M. J., Chaplin, M. J., Davis, L. A., Dagleish, M. P., Reid, H. W. 2005. Diagnosis of preclinical scrapie in samples of rectal mucosa. *Veterinary Record* 156: 846–847.

Spraker, T. R., Gidlewski. T. L., Balachandran, A., VerCauteren, K. C., Creekmore, L., Munger, R. D. 2006. Detection of PrP^{CWD} in postmortem rectal lymphoid tissues in Rocky Mountain elk (*Cervus elaphus nelsoni*) infected with chronic wasting disease. *Journal of Veterinary Diagnostic Investigation* 18: 553–557.

Wolfe, L. L., Spraker, T. R., Gonzalez, L., Dagleish, M. P., Sirochman, T. M., Brown, J. C., Jeffrey, M., and Miller, M. W. 2007. PrP^{CWD} in rectal lymphoid tissue of deer (*Odocoileus* spp.). *Journal of General Virology* 88: 2078–2082.

a) Agent: lidocaine & prilocaine
b) Vehicle: cream
c) Route: topical
d) Volume: 1-2 g
e) Dose: to effect
e) Frequency: twice
f) Duration (how long administered): n/a

Experimental Diet ..... ☐ Yes ☒No
If yes, describe:

Fluid collection ..... ☐ Yes ☒No
If yes, list following information for each:

Fluid:
Collection Site/Method:
Volume Collected:
Frequency:
Percent of total blood volume withdrawn:

20. Describe any adverse effects that may occur *secondary* to experimental agents, procedures or field manipulations: None anticipated. If adverse effects do occur, the field veterinarian will assess and decide on the best approach for treatment or euthanasia.

21. Methods to be used for monitoring animal well-being will include:
(Answer Y for all that apply)

a) Use of clinical scoring system ..... ☐ Yes ☒ No
Attach or provide IACUC SOP number:
Frequency and Duration: Daily for captive animals & at least every 2 weeks for free-ranging animals; duration ≥5 min.

b) Observation for changes in behavior, posture and activity ..... ☒ Yes ☐ No
Frequency and Duration: Daily for captive animals & at least every 2 weeks for free-ranging animals; duration ≥5 min.

c) Observation for pain and discomfort ..... ☒ Yes ☐ No
Frequency and Duration: Daily for captive animals & at least every 2 weeks for free-ranging animals; duration ≥5 min.

d) Observation of procedural area for local irritation/infection ..... ☒ Yes ☐ No
Frequency and Duration: Daily for captive animals & at least every 2 weeks for free-ranging animals; duration ≥5 min.

e) Observation for decreased activity/inability to move ..... ☒ Yes ☐ No
Frequency and Duration: Daily for captive animals & at least every 2 weeks for free-ranging animals; duration ≥5 min.

f) Assessment of daily food/water consumption ..... ☒ Yes ☐ No
Frequency and Duration : Daily for captive animals & at least every 2 weeks for free-ranging animals; duration ≥5 min.

g) Other (describe):

22. Analgesia
Will animals experience more than momentary pain/distress ..... ☒ Yes ☐ No
(If yes, complete below. If no, move to question #23)

a) Analgesic Drug:
b) Dose (mg/kg):
c) Route:
d) Frequency:
e) Duration:

The IACUC requires animals receive analgesia for 72 hours post surgery. **If no analgesic will be used to eliminate a potentially painful or distressful condition, provide justification**:

23. Overview of procedures to be conducted with animals.

a) Will any of the following occur?
If any yes answer is given, describe under item f below

a) Physical restraint greater than holding or transporting animals ..... ☐ Yes ☒ No
b) Use of paralytic drugs (must be scientifically justified) ..... ☐ Yes ☒ No
c) Unusual housing conditions ..... ☐ Yes ☒ No
d) Food or water deprivation other than pre-surgery ..... ☐ Yes ☒ No
e) Extreme environmental conditions ..... ☐ Yes ☒ No
f) Describe and justify any "yes" answer above:

b) Provide a brief description of experimental groups, key procedures, frequency and type of sampling, and endpoints. You can summarize if specific information is provided elsewhere, but *a response here is required.*

We will study three sub-populations in northeast Colorado, chosen from undeveloped, public land. There are several candidates for study identified as distinct units using cluster analysis based on radiotelemetry location

data. Each of these candidate sub-populations offers 11 years of data on CWD prevalence from ongoing surveillance. CWD prevalence among males averages 15-35% and is increasing exponentially; female prevalence is < 10% and appears static. Final choices of study areas will be based on pilot surveys of genetic composition, availability and duration of time series of data on sex and age composition and total census, and our ability to control access and hunting.

Mark-recapture data

During November of year one, we will capture 80 animals from each study population (total captures = 240) using aerial net gunning from a helicopter, a procedure widely used for capturing large mammals in open habitats.. We will initiate search from random starting points within each study area; thereafter deer will be captured as encountered. All captured animals will be tested for CWD using rectal biopsy, marked with a visible ear tag, fitted with a VHF radio collar equipped with a mortality sensor / transmitter. Tissue samples will be collected for genetic analysis. During November of years 2-5, we will recapture approximately 50 animals from each subpopulation and add 10 new marks (total captures for all three study areas ≅ 180). Aerial telemetry from a fixed wing aircraft will be used to direct the helicopter to marked animals to facilitate recapture. We anticipate that 300-320 individual animals will be marked.

Location data

Fifteen animals in each population, captured as described above, will also be fitted with global positioning system collars programmed to release from the animal approximately one year after deployment. These will be annually retrieved, GPS data will be downloaded, units will be refurbished with fresh batteries, and redeployed on new animals. In so doing we will accumulate a sample of 225 individual-years of observation by the end of the study.

Genetic measurements

We will collect tissues from all captured deer to examine the role of genetics in creating heterogeneity in transmission. Two measures will be used as covariates in the analysis described above. First, we will use a simple restriction fragment analysis (Jewell et al. 2005) to determine *PrP* genotypes of all captured deer. The *PrP* gene, including the ~250 amino acid proteins coded for by exon 3, has been described in a large number of vertebrates and is variable in at least seven cervid species. Thus, variation in the *PrP* gene in mule deer relative to other cervids is well established. We will test all individuals to determine their *S/F* genotypes, and genotypic information will be used in all population genetic analyses and as covariates in the analysis of sources of heterogeneity in transmission, described above. Second, all deer will be genotyped for a series of microsatellite markers and the control region of the mitochondrial DNA (mtDNA), which will allow: 1) calculation of relatedness among all captured deer; and 2) assessment of movement of males relative to females.

PART III—Training and approvals

24. Will animals or their wastes or experimental agents be, or possibly be:

a) Biohazardous (infectious agents or rDNA/transgenics) ..... ☐ Yes ☒ No

If yes, identify agents or rDNA use, describe potential risk to personnel/environment and risk management steps you've taken.

There is substantial evidence that CWD is not a risk to human health (reviewed by Novakofski et al. 2005), although such risks cannot be absolutely ruled out. Our studies will collect blood and rectal mucosa from wild animals; blood samples will be returned to the laboratory of Michael Antolin for DNA extraction. All personnel handling animals and tissue will wear latex gloves and will wash thoroughly after procedures are conducted.

We will not concentrate infectious material. No part of our work will expose animals to infectious material. All assays for CWD infection status will be conducted by the Colorado State University Diagnostic Laboratory. Conversation with Bill Mosely and verified by Robert Ellis confirmed that our proposed work does not require an IBC protocol.

Novakofski, J., M. S. Brewer, N. Mateus-Pinilla, J. Killefer, and R. H. McCusker. 2005. Prion biology relevant to bovine spongiform encephalopathy. Journal of Animal Science **83**:1455-1476.

Cite IBC approval number. NA
See website http://web.research.colostate.edu/ricro/ibc/ibc.aspx for information on required approvals.

b) Radioactive ..... ☐ Yes ☒ No
See website http://www.ehs.colostate.edu/WRad/Home.aspx or contact Jim Abraham, 491-3736 for information on required approvals.

c) Carcinogenic or chemically hazardous to humans or other animals (Y/N) ... . ☐ Yes ☒ No
Contact Environmental Health Services at 491-6745 for information on required approvals.

d) Will controlled drugs (including HCG and Ketamine) be used (Y/N) .... ☐ Yes ☒ No
If yes, list whose drug cabinet will be accessed.
See website http://web.research.colostate.edu/ricro/drc/drc.aspx for information.

25. Documentation of Training

a) CSU "Handbook for Investigators Using Laboratory Animals" read/provided to staff ☒ Yes ☐ No
Details:

b) Specific or targeted training performed on site .... ☐ Yes ☒ No
Describe (who, by whom, topics, etc.): Personnel involved with capture & handling already have extensive field experience.

c) PI has a written description of SOPs available .... ☒ Yes ☐ No
Specify location of SOPs related to the species used in this project: CDOW ACUC

d) Pertinent training/education of people handling animals .... ☒ Yes ☐ No

e) Other (describe):

PART IV—Euthanasia

26. Will euthanasia be performed? .... ☐ Yes ☒ No

If yes, move to question 27. If no, complete following information to specify what will happen to animals at study end.

a) Adoption .... ☐ Yes ☒ No
b) Transfer to other studies ...(all captive deer are & will remain property of state of CO) ..... ☒ Yes ☐ No
c) Sold at auction (hoof stock only) .... ☐ Yes ☒ No
d) Released into home territory .... ☒ Yes ☐ No

27. Describe experimental endpoints or clinical signs that will determine when euthanasia will be performed.
(Death is not an acceptable endpoint unless extensively justified). Describe euthanasia method to be used should unanticipated complications arise and euthanasia becomes necessary.
None anticipated; collars will drop off automatically from free-ranging deer and will be removed by hand from captive deer.

Endpoints: In the rare cases that (<1% of captures, based on our experience) that deer are severely injured during capture and handling, they will be deeply anesthetized and then euthanized with KCl (400-1000 mEq IV). if an injured deer cannot be handled (e.g., deer that fractures its leg during a failed induction), then it will be killed with a gunshot to the head or chest with a ≥0.22 magnum caliber rifle or pistol.

28. Euthanasia method/agent:
Should be consistent with guidelines published by the AVMA Panel on Euthanasia. See http://www.avma.org/issues/animal_welfare/euthanasia.pdf.

a) Species:
b) Agent/Method:
c) Dose (mg/kg):
d) Route:

29. Documentation of Training.
Please list all personnel who will be associated with this protocol and the roles in which they will be participating (Table 29 a). For each person listed you will be required to complete training documentation (Table 29 b).

Table 29a—All Personnel Associated with this Protocol

Role	NAME, Degree, Certification	Is this person Key Personnel (e.g. have administrative duties on this protocol, including receiving email notifications of protocol status)?	Will this Person be Performing Procedures? (e.g. animal handling, injections, anesthesia, surgery, euthanasia). If yes, please complete Table 29b for this individual.	Will this Person be Ordering Animals?	Which Individual Species is this individual authorized to use on this protocol?
PI	N. T. Hobbs, PhD	yes	no	no	
Co-PI	Michael Miller, D.V.M., Ph.D.	yes	yes	no	mule deer
Staff Roster					

Comments:

Table 29b

Training Record for: M. Miller

For each staff member performing procedures as indicated in Table 29a, provide the training and experience for the procedures listed for each species.

Please attach additional sheets as needed.

Procedures	Species	Method/Route	Experience	Training Required
Injections	Mice/Rats			
	mule deer	DVM	>25 years	none
Blood Collection	Mice/Rats			
	mule deer	DVM	>25 years	none
Anesthesia	Mice/Rats			
	mule deer	DVM	>25 years	none
Euthanasia	Mice/Rats			

	mule deer	DVM	>25 years	none
Restraint and Handling (Specify devices or methods)	Mice/Rats			
	mule deer	DVM	>25 years	none
Surgery (List specific procedures in Methods column)*	Mice/Rats			
	Other (specify)			
Animal ID (e.g. ear punch or tag, microchip, etc.)	Mice/Rats			
	Other (specify)			
Other (Describe procedure in the Methods column)	Mice/Rats			
	Other (specify)			

*Identify the required training for surgery by listing one or more of the following requirements:

a. Completed AN550, surgery course
b. Obtained DVM degree
c. Has previous experience with this procedure. Provide a description.
d. Continuing Education e.g. in-service, seminars

I understand that changes in the approved protocol must be submitted in writing to the IACUC as a protocol amendment and approved by the IACUC prior to implementation. Such changes include, but are not limited to: species, animal numbers, animal-related procedures, animal restraint, food/water deprivation, euthanasia, PI, research staff, and the like. Minor changes can be emailed to Bill.Moseley@research.colostate.edu for review by one or more IACUC members; significant changes (e.g. a large increase in animal numbers, adding an invasive procedure) usually require a new A-100 be submitted for review by the IACUC at its next regularly scheduled meeting.

ASSURANCE STATEMENT

Please read the following before you sign this form:

As Principal Investigator, I:

Assure that these studies do not unnecessarily duplicate previous experiments.

Will abide by all relevant portions of the Public Health Service Policy and the USDA Animal Welfare regulations and guidelines concerning activities involving animals. For full text, see http://web.research.colostate.edu/ricro/acuc/acuc.aspx.

Agree to furnish IACUC with any relevant information on animal use it requests.

Assume responsibility for the ethical conduct of this project to protect the welfare of the animals.

Agree to attend and have my key personnel attend appropriate IACUC training opportunities.

Assure that personnel conducting animal procedures will be appropriately qualified and trained in these procedures. Assure that all individuals performing surgery under this protocol have been authorized by the IACUC to do so, as required by IACUC.

Understand that my signature acknowledges that I have reviewed this form and am responsible for this project.

Principal Investigator signature _____ **Date** July 8, 2008

As Department Head, I understand that my signature on this form acknowledges that I have read this application and approve of this research.

Department Head signature _____ **Date** 7/7/08

Note: Alternate faculty signature for Department Head must be specifically delegated to another faculty member by the Department Head in advance.

With your IACUC application, provide just ONE copy of the complete funding proposal. This is to meet federal requirements that each IACUC approval be verified to involve the research included in the funding proposal.