PROTOCOL
ARS Form
USDA

For Official Use Only

Protocol # ARS-2019-827
December 18, 2019

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

Protocol Type: ARS Form

Approval Period: 11/25/2019-11/24/2022

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Personnel Information * * *

PRINCIPAL INVESTIGATOR

The Principal Investigator can view, edit, and submit protocol.

Principal Investigator

Name*	Department
Greenlee, Justin	Virus and Prion Research Unit
Email*	**Phone**
Justin.Greenlee@ARS.USDA.GOV	515-337-7191

CO-PRINCIPAL INVESTIGATOR(S)

Co-Principal Investigator(s)

Name	Department	Email
[redacted]	[redacted]	[redacted]

OTHER PERSONNEL

Other Personnel

Name	Department	Email
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Emergency Information:

List at least two contacts. If more than two are listed, the first two entered will appear on door card.

Emergency Contact		After Hours Number
Name	Extension	
[redacted]	[redacted]	[redacted]

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* * * Species * * *

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

Species to be Used

Species Common Name	Proposed Housing Facility	Maximum number of animals requested for this species for this housing location (total 1 year)
Deer	As Assigned by ARU	120

Species to be Used

1. **Species Common Name*** Deer
2. **Scientific Name** Cervidae
3. **Strain/Breed** Whitetail
4. **Animal Sex*** Either
5. **Age Range** 0 - 12 Year(s)
6. **Weight Range** - Kg(s)
7. **Proposed Housing Facility*** As Assigned by ARU
   4 B/C
8. **USDA Pain Category (Choose all that will apply. Enter the total number of the species to be used in each Pain Category. If animals will be used in more than one category, enter the number in the higher category.***

	Pain Category B	
	Pain Category C	
	Pain Category D	
X	**Pain Category E**	120

9. **Maximum number of animals requested for this species for this housing location (total 1 year)*** 120
10. **State whether enrichment should be provided (YES or NO). If no, give a scientific justification why not.** Yes
11. **Have any of the animals undergone procedures prior to being used on this protocol?** N

    Please specify which animals underwent procedures, what procedures were performed, and where those procedures were performed.

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* * * Agent Information * * *

Agent Information - Collaboration and Safety Concerns

1. **Biological Material / Human or Animal Product(s) / Infectious Agent(s)***

   Are you using biological material, human or animal products, or infectious agents? Y

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

Biological Material / Human or Animal Product(s) / and /or Infectious Agent

Specify Material or Agent	Spread by
Chronic wasting disease prion	exposure to infected deer or contaminated environments

Biological Material / Human or Animal Product(s) / and /or Infectious Agent

1.a.	Specify Material or Agent(s)*	Chronic wasting disease prion
1.b.	Strain/Type of Agent* prion	
1.c.	Spread by*	exposure to infected deer or contaminated environments
1.d.	Route of Administration*	Oronasally
1.e.	Is this agent a known pathogen to	Animal
1.f.	Is this agent(s) a select agent?	N
1.g.	Does this protocol use recombinants/Genetic Engineering Organisms? **If yes, provide IBC number** **Expiration date**	N

2. Other Agents or Substances* N

In this section please specify any additional substances such as hormones, novel antibiotics, cytokines, inhibitors, non-toxic irritants, etc., that are not covered above in section.

3. Entry Procedures

Entry Procedures(for the agent)

X	Complete Clothes Change		Lab Coat
X	Boots/Shoe Covers	X	Face Shield
	Shower In		Safety Glasses
X	Gloves		Surgical or Dust Mask
	Hair Net		Tyvek
X	Other(fill out textbox below)		Respirator(state type in textbox below)
	When mist is produced (during pen washing or inoculation), a faceshield or surgical/procedural mask should be worn to prevent splashing into the nose or mouth. N95 respirators will be available to all personnel. Change clothes in.		

4. Exit Procedures

Exit Procedures(for the agent)

X	Remove Clothing Worn in Unit	X	Disinfect Carry Out Items

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X	Wash Hands & Forearms	X	Shower Out
	Other(fill out textbox below)		Wash/Disinfect Footwear

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* * * Rationale * * *

USDA Protocol ID
(for office use only)

Official Project Title

Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

Study Objectives

1. Objectives and Significance

a. **Provide a brief synopsis of the research project covered by this protocol and its overall objective(s).***

Most white-tailed deer appear to be highly susceptible to the chronic wasting disease (CWD) agent. Deer with specific amino acid combinations (polymorphisms) in their prion protein have been shown to be unrepresented in the CWD positive cases from premises with large numbers of positive deer. This purpose of this study is to test for potential resistant genotypes of deer through direct inoculation and exposure to infected deer of known susceptible genotypes and characterize shedding of the abnormal prion protein in deer that become infected.

b. **Why is the study important to human or animal health, the advancement of knowledge, or the good of society?***

Since originating on a single premises, CWD has spread worldwide. Identifying resistant genotypes of deer would provide a management tool for reducing cases of CWD on farms and in the wild.

2. Rationale for Use of Animals

a. **How will the use of animals help you accomplish the project goal(s)? Explain why those goals could not be achieved using in vitro or computer models.***

Using deer for this experiment will allow us to better understand CWD transmission and the potential to use selective breeding as a control measure. Using deer is essential to reaching the goals of this project. In vitro and computer models do not have the complexity required to evaluating prion agent resistance in the natural host.

b. **Why are the species you have selected the most appropriate for these studies?***

White-tailed deer (WTD) are the most appropriate species for these studies because more cases of CWD have been identified in WTD than any other cervid species, WTD are the most widely distributed species of deer in North America, and amino acid changes in the WTD prion protein are well-described.

c. **Indicate how group sizes (number of animals per project) were determined. Justification for these numbers using an appropriate statistical assessment such as a Power Analysis is expected. If a Power Analysis is not appropriate (e.g., pilot studies, tissue protocols, etc.) provide a detailed description of how the requested number of animals was determined. Be sure to include descriptions of the groups (e.g. Control, treatment, etc.) and the numbers of animals included in each group. ***

There are 3 polymorphic sites in the prion protein of white-tailed deer that we wish to assess in this protocol: codons 95, 96, and 226. Studies covered in this protocol will require assessment of up to 10 genotypes of deer. Numbers requested for this experiment are for two purposes: 1) assess susceptibility after direct inoculation (60; 6 deer of up to 10 different genotypes) and 2) assess susceptibility of these genotypes after cohousing with infected deer to model natural exposure (60, which requires direct inoculation of up to 20 deer of susceptible genotypes and exposure by co-housing to up to 40 deer of potentially resistant genotypes). We plan to use 5-6 deer per group similar to previously published studies (Greenlee, J. J., Smith, J. D. and Kunkle, R.

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A. White-tailed deer are susceptible to the agent of sheep scrapie by intracerebral inoculation. Vet Res. 42 (1): 107. 2011).

Testing the potential of the CWD agent to transmit to deer after oronasal exposure will require up to 60 deer (up to 6 deer per each of 10 genotypes). All exposure will be by the intranasal/oral route.

Testing the potential of the CWD agent to transmit to deer after cohousing with infected deer will require up to 60 deer (up to 6 deer per group, each group will contain 2 deer infected deer and 4 of the each of the 10 genotypes being tested). The infected deer will be exposed to CWD prions by the intranasal/oral route and cohoused with deer being assess for susceptibility.

All groups will be similarly assessed for shedding of CWD prions.

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* * * Procedures * * *

Biopsy

1. **Procedure Type:** Biopsy
2. **Brief Description:** rectal mucosal biopsy with use of topical lidocaine lubricant
3. **Species:** Deer (As Assigned by ARU)
4. **USDA Pain/Distress Category:** D
5. **Maximum number of animals to be used in this procedure:** 120
6. **Please click 'save' and then address all of the questions for each tab that will appear below. Incomplete or missing details will prevent the protocol from being submitted to the IACUC for review.**

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

Deer infected with the agent of chronic wasting disease accumulate abnormal prion protein in lymph nodes and other peripheral structures with similar populations of lymphoid cells such as third eyelid, tonsil, and the Peyer's patches of the small intestine. Nodules of lymphoid tissue appropriate for testing for abnormal prion protein by immunohistochemistry also are present in the rectal lining at the rectal-anal junction. A simple biopsy procedure is used to collect a sample of this tissue. Briefly, a speculum is inserted into the rectum with use of 0.2% lidocaine in the lubricant; forceps are used to elevate one of the small folds of

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rectal mucosa immediately inside the rectal-anal junction; scissors are used to remove a dime to quarter-sized piece of mucosa, which is processed for microscopic examination and immunohistochemical staining. Previous experiences with elk and sheep suggest that this procedure will be well tolerated. Complications are rare, and there is minimal evidence of biopsy detectable two weeks post-procedure. The biggest risk with performing this procedure in white-tailed deer is associated with handling the deer, which can lead to injured deer if physical restraint is not adequate. This procedure could yield very valuable data when assessing transmission of CWD to sentinel deer. If we choose to use this technique, we ensure that there is adequate staff on hand to manipulate the deer as quickly, quietly, and safely as possible. Multiple biopsies (up to every two months) may be obtained over the course of the experiment by sampling at different locations around the circumference of the rectum. Biopsies will be performed by the PI or other personnel trained in the technique.

2. **Please list and describe any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.**

Immediately after the procedure, there may be a small amount of bleeding from the rectum. No long term clinical effects or consequences are expected.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Deer will be observed immediately after the procedure and daily during normal observation periods.

4. **What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?**

No signs requiring euthanasia are expected as a consequence of this procedure.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

1. **Parameters used to monitor and ensure appropriate anesthetic depth.**

Anesthetic Agents

Agent Name	Dosage (in mg/kg if possible) AND Volume of Administration (when applicable)	Route
Lidocaine	2-3 ml of 0.2% lidocaine in lubricant	Topical

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

* * * Perioperative Care * * *

Perioperative Care

Describe what parameters will be monitored during surgery to ensure proper analgesia.

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

1. Recovery Location - Facility or Building Name
2. Room Number
3. Personnel Responsible for Monitoring Recovery
4. What parameters are monitored to assess recovery?
5. Recovery - What is the duration and frequency of the monitoring?
6. Post-recovery - What is the duration and frequency of the monitoring ?

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* * * Pharmaceuticals * * *

Pharmaceuticals

Animal Identification

1. **Procedure Type:** Animal Identification

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

2. **Brief Description:** ear tags will be applied, this may require making an ear punch
3. **Species:** Deer (As Assigned by ARU)
4. **USDA Pain/Distress Category:** C
5. **Maximum number of animals to be used in this procedure:** 120

6. **Please click 'save' and then address all of the questions for each tab that will appear below. Incomplete or missing details will prevent the protocol from being submitted to the IACUC for review.**

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

We will use ear tags for animal identification as each room may contain up to 6 deer that look very similar. An ear punch tool will be used to remove a piece of ear skin/cartilage and ear tag pliers will be used to apply an ear tag. Preforming a hole with the punch greatly reduces complications due to infection.

2. **Please list and describe any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.**

No clinical effects are anticipated from this procedure.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Deer will be monitored immediately after the procedure, upon returning to their room, and during daily observation periods throughout the experiment.

4. **What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?**

No consequences severe enough to warrant euthanasia are expected from this procedure.

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* * * Pharmaceuticals * * *

Pharmaceuticals

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

Challenge

1. **Procedure Type:** Challenge
2. **Brief Description:** oronasal inoculation
3. **Species:** Deer (As Assigned by ARU)
4. **USDA Pain/Distress Category:** C & E
5. **Maximum number of animals to be used in this procedure:** 80

6. **Please click 'save' and then address all of the questions for each tab that will appear below. Incomplete or missing details will prevent the protocol from being submitted to the IACUC for review.**

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

Inoculum (usually 1 ml of 10% tissue homogenate) is given by slightly elevating the deer's nose and inserting a 3 ml syringe without a needle into the nasal cavity. Inoculum is slowly delivered over several seconds and the nose remains elevated until there is no evidence of liquid near the nares and/or the deer swallows.

2. **Please list and describe any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.**

No immediate clinical effects or changes are expected from this procedure. Clinical signs of weight loss, altered gait, tremor, or behavioral change could occur 18-60 months post inoculation. For some prion agent isolates we are unsure how many animals may develop clinical disease.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Deer will be monitored at the time the inoculum is delivered and during daily observation periods throughout the experiment.

4. **What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?**

Clinical signs as a result of the inoculum may occur 18-60 months later and include altered gait, tremor, or behavioral change, which may occur in conjunction with apparent weight loss. Animals will be necropsied at onset of definitive clinical signs (see above) and tissues will be examined for the presence of abnormal prion protein accumulation. Recovery is not anticipated, so animals will be euthanized when assessments

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reveal unequivocal signs of prion disease.

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* * * Pharmaceuticals * * *

Pharmaceuticals

Blood Collection (non-terminal)

1. **Procedure Type:** Blood Collection (non-terminal)
2. **Brief Description:** IV blood collection from jugular vein
3. **Species:** Deer (As Assigned by ARU)
4. **USDA Pain/Distress Category:** C
5. **Maximum number of animals to be used in this procedure:** 120

6. **Please click 'save' and then address all of the questions for each tab that will appear below. Incomplete or missing details will prevent the protocol from being submitted to the IACUC for review.**

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

Blood will be periodically collected for use in developing new assays for prion disease diagnostics and for confirming deer genotypes. Blood collection will be performed from the jugular vein in accordance with SOP ARU 0300. The expected schedule for collections is up to every two months. The anticipated volume of collection is 30-50 mL. Larger volumes (not to exceed 200 ml) may be collected from animals that reach the clinical stage of disease.

2. **Please list and describe any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.**

No clinical effects are expected from this procedure.

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3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Monitoring will occur during and after the procedure, upon returning the deer to their rooms, and during daily observation periods throughout the experiment.

4. **What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?**

No changes warranting euthanasia are expected from this procedure.

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* * * Pharmaceuticals * * *

Pharmaceuticals

Biopsy

1. **Procedure Type:** Biopsy
2. **Brief Description:** skin biopsy
3. **Species:** Deer (As Assigned by ARU)
4. **USDA Pain/Distress Category:** C
5. **Maximum number of animals to be used in this procedure:** 120

6. **Please click 'save' and then address all of the questions for each tab that will appear below. Incomplete or missing details will prevent the protocol from being submitted to the IACUC for review.**

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

A skin biopsy may be taken to assess for CWD prions. While deer are physically restrained for periodic sampling, an ear marking pliers will be used to to take a small (3x5x5 mm) wedge shaped piece of skin from the ear.

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2. **Please list and describe any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.**

Immediately after the procedure, there may be a small amount of bleeding from the margins of the excised wedge of ear. No long term clinical effects or consequences are expected.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Deer will be observed immediately after the procedure and daily during normal observation periods.

4. **What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?**

No signs requiring euthanasia are expected as a consequence of this procedure.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

1. **Parameters used to monitor and ensure appropriate anesthetic depth.**

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

* * * Perioperative Care * * *

Perioperative Care

Describe what parameters will be monitored during surgery to ensure proper analgesia.

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

1. Recovery Location - Facility or Building Name
2. Room Number
3. Personnel Responsible for Monitoring Recovery
4. What parameters are monitored to assess recovery?
5. Recovery - What is the duration and frequency of the monitoring?
6. Post-recovery - What is the duration and frequency of the monitoring ?

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* * * Pharmaceuticals * * *

Pharmaceuticals

Sample Collection

1. **Procedure Type:** Sample Collection

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

2. **Brief Description:** collection of saliva, feces, urine
3. **Species:** Deer (As Assigned by ARU)
4. **USDA Pain/Distress Category:** C
5. **Maximum number of animals to be used in this procedure:** 120

6. **Please click 'save' and then address all of the questions for each tab that will appear below. Incomplete or missing details will prevent the protocol from being submitted to the IACUC for review.**

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

These excretions/secretions will be assessed for the presence of CWD prions. Samples will be collected at intervals of up to every two months while deer are physically restrained in a squeeze chute. Saliva will be collected with a transfer pipette or by using a collection system such as the "Super SAL" kit that collects saliva on an absorbent pad. Feces will be collected during evacuation or digitally prior to performing rectal biopsy (see separate section). Urine will be collected by free catch, if possible, while deer are restrained.

2. **Please list and describe any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.**

No long term clinical effects or consequences are expected.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Deer will be observed immediately after the procedure and daily during normal observation periods.

4. **What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?**

No signs requiring euthanasia are expected as a consequence of this procedure.

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* * * Pharmaceuticals * * *

Pharmaceuticals

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

* * * Literature Search * * *

1. Literature Search for Alternatives to the Use of Animals AND Alternatives to Painful or Distressful Procedures.

Search Data

Search Range From	Search Range To
1965	2019

Search Data

1.a.	Search Range From*	1965	(YYYY)
1.b.	Search Range To*	2019	(YYYY)
1.c.	Search Date*	09/24/2019	(MM/DD/YYYY)

Note: Because this is a search for alternatives to painful or distressful procedures, you are advised to use the word "alternative" as a search term along with words that describe the painful procedures described in this protocol.

1.d.	Keywords*	chronic wasting disease, deer, transmission, pathogenesis, alternative, animal use

1.e. Databases Searched*

	Agricola Database	X	Pubmed
X	Google Scholar		Digitop
	Other		

2. Based on your literature search, are there refinements, replacements, reductions or alternatives that would be compatible with your experimental design? If "yes", please explain why you are not using the alternative(s).

No alternative to the planned procedures were identified.

3. Duplication of Results

X **I attest that the proposed animal activities do not unnecessarily duplicate previous experiments, whether my own or another investigator's experiments.***

4. Alternatives for Category E Procedures

For Category E procedures, explain why pain relieving drugs or other ameliorative treatments cannot be used to alleviate pain/distress.

Drugs will be used to alleviate pain and distress that occurs during procedures or due to intercurrent disease. Animals are requested in category E due to the potential that they will exhibit clinical signs as a result of being inoculated with a prion disease agent. Currently, there are no known treatments for prion disease.

5. Previous Protocol

List previous protocols. If none, state "NA" or "none".

NA

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6. Unforeseen Events

X **If unforeseen events such as injury or illness unrelated to experimental procedures occur, a veterinarian will be contacted as soon as possible for appropriate treatment or the animal will be euthanized. ***

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* * * Sequence and Timing * * *

Sequence and Timing

1. **Please describe the sequence and timing of all the manipulations for each group of animals. Also include numbers used for each group of experiments, drugs and substances administered in each group, the time between procedures, and experimental endpoints. Use enough detail to allow reviewers to understand what each animal may undergo. Please separate paragraphs with a blank line.**

Deer will be assigned to one of two groups: 1) direct inoculation of potentially resistant genotypes (60 deer) or 2) exposure of deer of potentially resistant genotypes (4/room) to CWD by co-housing with inoculated animals of the most common/susceptible genotype (2/room) (60 total, 20 inoculated, 40 exposed by co-housing). Animals will be inoculated (n=80) and housed in groups or cohoused with non-inoculated deer (n=40). All deer will be observed daily for clinical signs. Prior to inoculation and periodically (up to every two months) deer (n=120) may be physically restrained for collection of blood, urine, and feces; rectal mucosal biopsy (with use of 0.2% lidocaine in the lubricant); and skin biopsy. Deer will be euthanized and necropsied when definitive signs of CWD are present such as altered gait, tremor, or behavioral change, which may occur in conjunction with weight loss. Clinical signs are expected 18-60 months post-inoculation. If inoculated animals fail to develop clinical signs 60-84 months post-inoculation, the experiment will be ended and tissues will be collected for analysis.

2. **Optional Flow Chart - If the protocol involves more than one procedure (i.e., simple euthanasia and tissue harvest), please submit a procedures flow chart with this protocol. The flow chart should illustrate/include in chronological order all the procedures that the animals will undergo starting with their arrival on the protocol and ending with their euthanasia. Indicate the timeline for the events (i.e., if animals are involved in multiple procedures, note the time period between procedures). Please use the Add feature to attach the document in the Attachments section of Protocol Information.**

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* * * Husbandry * * *

1. Special Husbandry or Care

List any special or unusual requirements for the care of animal subjects and who will provide this care (e.g., special diet or supplements, special water, altered light cycles, etc.). Indicate N/A, if not applicable.

These deer should not be fed any feed containing animal ingredients.

Non-Standard Experimental Requirements

2. Food or Fluid Restriction X None

Note: This does not include pre-surgical fasting.

Food or Fluid restriction

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

Species	Food Restriction	Duration and Frequency of Restriction	Fluid Restriction	Duration and Frequency of Restriction	Reason for Restriction
Deer (As Assigned by ARU)					

3. Describe the health monitoring procedures (e.g., body weight, blood urea nitrogen, urine/fecal output, food/fluid consumed), frequency of checks, and the method of ensuring adequate nutrition and hydration during the regulated period.

4. Restraint of Conscious Animals X None

Note: Include only prolonged restraint; brief restraint or restraint of anesthetized animals need not be described.

Restraint of Conscious Animals

Species	Type of Restraint	Please describe acclimation to restraint	Duration and frequency of restraint
Deer (As Assigned by ARU)			

5. Please justify the need for the restraint prolonged and describe the monitoring procedures and criteria for removing animals that do not adapt or acclimate to the restraint.

6. Non-standard housing requirements X None

Species	Cage/Pen Size	Cage Sanitation Interval	Wire-bottom rodent cages or grids	Animals outside dedicated animal housing for greater than 12 hours	Exemption from social housing or enrichment
Deer (As Assigned by ARU)					

7. Provide a description of the non-standard housing and justify why it is needed for your experimental design.

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* * * Euthanasia * * *

Protocol Title: Examination of deer with prion protein amino acid polymorphisms for potential resistance to exposure to the chronic wasting disease (CWD) agent.

Euthanasia

Method of Euthanasia	Dosage (in mg/kg if possible) or inhalation or immersion agent, the concentration	Species	Route of Administration
Barbiturates (Sodium Pentobarbital)	As per label instructions or as directed by an ARU veterinarian	Deer (As Assigned by ARU)	Intravenous

Euthanasia

Species	Deer (As Assigned by ARU)
Method of Euthanasia Primary	Barbiturates (Sodium Pentobarbital)

*** * * Guidelines * * ***

Mandatory (view and check Yes)

Respirator Fit Test & Training — AGREE

Non-Mandatory (view those relevant and check Yes)

Unforeseen Events — AGREE

*** * * Certifications * * ***

STATEMENT CONCERNING THE CARE AND USE OF LABORATORY ANIMALS

The NADC has on file with NIH's Office of Laboratory Animal Welfare (OLAW) a written Assurance of Compliance which commits this institution to following the standards established by Public Health Service (PHS) Policy. As part of the Assurance, NADC has established an institutional animal care and use committee (IACUC) to review, require changes to, and/or grant approval to research protocols or teaching projects involving the use of vertebrate animals. The IACUC is also charged with ascertaining if research proposals are consistent with the "US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training"; the Guide for the Care and Use of Laboratory Animals, National Research Council; the Animal Welfare Act/Regulations, and the Guide for the Care and Use of Agricultural Animal in Research and Teaching, Federation of Animal Science Societies. These regulatory documents describe the minimal standards that must be met for humane care and treatment of research animals to assure that animals do not suffer unnecessary discomfort, pain, or injury, and that animals receive proper care and husbandry. Research animals must be cared for and used in a manner that complies with the above documents to protect current and future sponsored support.

ANIMAL USER CERTIFICATION

All personnel listed on this protocol are responsible for reading the above statement and will be held responsible for adhering to all regulations therein, and agree to make written notification in the form of a protocol amendment to the Institutional Animal Care and Use Committee (IACUC) of any proposed changes in the animal experimentation protocol for review and approval prior to proceeding with any animal experimentation.

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animal experimentation.

All personnel working with animals on this protocol:

- must enroll in the NCAH Occupational Health Program to work with animals;
- will be held responsible for pursuing appropriate training; and
- must adhere to the terms and condition of this protocol as approved by the IACUC.

X The Principal Investigator has read and agrees to abide by the above obligations.