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# Using White-tailed Deer (*Odocoileus virginianus*) in Infectious Disease Research

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**Between 1940 and 2004, more than 335 emerging infectious disease events were reported in the scientific literature. The majority (60%) of these events involved zoonoses, most of which (72%) were of wildlife origin or had an epidemiologically important wildlife host. Because this trend of increasing emerging diseases likely will continue, understanding the pathogenesis, transmission, and diagnosis of these diseases in the relevant wildlife host is paramount. Achieving this goal often requires using wild animals as research subjects, which are vastly different from the traditional livestock or laboratory animals used by most universities and institutions. Using wildlife in infectious disease research presents many challenges but also provides opportunities to answer questions impossible to address by using traditional models. Cervid species, especially white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and red deer (*Cervus elaphus*), are hosts or sentinels for several important pathogens, some of which are zoonotic. The long history of infectious disease research using white-tailed deer, conducted at ever-increasing levels of sophisticated biosecurity, demonstrates that this type of research can be conducted safely and that valuable insights can be gained. The greatest challenges to using wildlife in infectious disease research include animal source, facility design, nutrition, animal handling, and enrichment and other practices that both facilitate animal care and enhance animal wellbeing. The study of *Mycobacterium bovis* infection in white-tailed deer at the USDA's National Animal Disease Center serves to illustrate one approach to address these challenges.**

**Abbreviations:** NADC, National Animal Disease Center; SCWDS, Southeast Cooperative Wildlife Disease Study; WTD, white-tailed deer.

## Introduction

The One Health Initiative emphasizes the interdependency of human, livestock, and wildlife health. Specifically, the initiative asserts that the physical health of humans, livestock and wildlife are linked through shared diseases.[11] Between 1940 and 2004, more than 335 emerging infectious disease events were reported in the scientific literature. The majority (60%) of these events involved zoonoses, most of which (72%) had an epidemiologically important wildlife host.[48] More than 90% of animal-related research at universities and other institutions involves traditional laboratory animals (that is, rats and mice).[64] Today, understanding the pathogenesis, transmission and diagnosis of emerging diseases often requires research involving wild animals, either free-ranging or captive, that are vastly different from laboratory animals or traditional livestock.

Cervid species, specifically white-tailed deer (WTD; *Odocoileus virginianus*) red deer (*Cervus elaphus*), and elk (*Cervus canadensis*), are important hosts for several zoonotic pathogens (for example, *Brucella abortus*, *Mycobacterium bovis*, hepatitis E virus).[74,76,107] These species also serve as sentinels for other zoonotic pathogens (for example, enterohemorrhagic *Escherichia coli*, *Yersinia enterocolitica*, *Listeria monocytogenes*).[27] Furthermore, some diseases such as brucellosis and tuberculosis are transmitted between cervids and livestock, making these important diseases at the wildlife–livestock–human interface. Experimental infection of cervids with zoonotic pathogens requires specialized facilities and practices to prevent pathogen release and to ensure personnel safety. In addition, pathogens such as *B. abortus* are considered biologic select agents and require intense biosecurity measures beyond standard practices.[4]

National biosafety guidelines categorize infectious agents into 4 ascending levels of risk (Figure 1). These designations are based on the pathogen's ability to infect and cause disease in humans or animals, severity of disease, and availability of preventive or therapeutic options.[118] These risk criteria are used to define corresponding biosafety levels of physical containment. Each of the 4 biosafety levels of containment describes the level of protection in terms of the practices, equipment, and facilities necessary for handling an agent of the corresponding risk level. These criteria also apply to the housing of animals infected with such agents. In situations where highly infective agricultural agents and large animals such as cows, pigs, bison, and deer are used, requirements beyond typical BSL3 practices are required. This advanced BSL3 designation is known as BSL3Ag.[118]

The following paragraphs describe published research using white-tailed deer. Some of the reported studies were conducted prior to the formal introduction of risk factors and biosafety levels of containment. As such, the descriptions of research facilities are those used at the time and are not necessarily facilities that would be appropriate today.

## Infectious Disease Research Involving WTD in BSL1 Environments

Pre1990 studies with WTD included infection trials with *Leptospira pomona* in 1962, *Salmonella meleagridis* in 1970, *Anaplasma marginale* in 1971, Venezuelan equine encephalomyelitis virus in 1972, *Fasciola hepatic in 1974*, *Fascioloides magna* in 1979, Jamestown Canyon and Keystone viruses in 1979, malignant catarrhal fever in 1981 and 1982, and *Mycobacterium avium* subsp.

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| Risk group | Description of agent | Examples |
|---|---|---|
| 1 | Not associated with disease in healthy humans or animals. | *Mycobacterium bovis* vaccine strain bacillus Calmette and Guerin (BCG) |
| 2 | May cause disease but is rarely serious and therapeutic options are available. | Epizootic hemorrhagic disease virus<br>Bluetongue virus (non-exotic)<br>*Mycobacterium avium* subsp. *paratuberculosis* |
| 3 | May cause serious disease (possibly lethal) and therapeutic or preventative options are available. | Bluetongue virus (exotic)<br>*Brucella abortus*<br>*Mycobacterium bovis* |
| 4 | Likely to cause serious disease (possibly lethal) and therapeutic or preventative options are not available. | Venezuelan Equine Encephalitis virus |

**Figure 1.** Recommended risk group classifications and examples of agents experimentally administered to WTD.

*paratuberculosis* in 1983[20,40,53,93,98,115-117] (Figure 2). Descriptions of containment facilities for each of these studies generally are not provided in the literature or are only minimally described; therefore, the animals can be assumed to have been housed in outdoor pens consistent with BSL1 containment. The study using *Anaplasma marginale* was done at a field laboratory operated by USDA in Nuevo Laredo, Tamaulipas, Mexico—presumably as a precaution given that the tick vector (*Boophilus annulatus*), although endemic in Mexico, was essentially eradicated from the United States at that time. Research on chronic wasting disease (CWD) at the Colorado Division of Wildlife's Wildlife Research Center from 2010 to 2013 housed CWD-inoculated deer in—presumably—outdoor "biosecure paddocks." During these studies, some deer were held for short periods of time in metabolic cages for urine and feces collection.[45,60,99]

## Infectious Disease Research Involving WTD in BSL2 Environments

The Southeastern Association of Fish and Wildlife Agencies founded the Southeast Cooperative Wildlife Disease Study (SCWDS) in Athens, GA, in 1957 to examine the cause of widespread die-offs of WTD. Objectives—then and now—are to determine the causes of disease in wildlife, effects of disease on wildlife populations, and interrelationships between wildlife, domestic livestock, and human infectious diseases. At the SCWDS, experimental infection trials in BSL2 type containment have included such notable pathogens as *E. coli* 0157:H7;[19] epizootic hemorrhagic disease virus,[23-26,88,89,100,103,104,106] bluetongue virus,[41,42] and multiple agents of anaplamosis,[65,108,109] borreliosis,[54,63,73] and ehrlichiosis[8-10,112,122,123] (Figure 2). For the past 50 years and longer, the SCWDS has been a leader in the development of experimental biology approaches for the study of infectious diseases in WTD.

Experimental infection studies with endemic strains of bluetongue virus were performed under BSL2 containment as early as 1967 at the University of Wisconsin (Madison, WI) and later at the SCWDS in the mid 1990s[41,106,114] (Figure 2). Richard E Shope demonstrated the viral etiology of epizootic hemorrhagic disease in WTD and detailed the pathologic manifestations of the disease.[102] Biocontainment for experimental infection trials performed by Shope at the Rockefeller Institute (Trenton, NJ) consisted of individual pens on a cement floor deeply bedded with straw or hay in a sturdy wooden frame lined with a 14-gauge welded wire of 2×1-in. mesh covered with a plastic "insect-proof" mesh screen. Studies with a California serovar of bluetongue virus (BTV8) at the University of Wisconsin used similar biocontainment measures, described as "a Rockefeller-type isolation building."[114] These early studies by Shope provided a framework for experimental biology approaches using WTD. Fletch and Karstad extended Shope's findings by demonstrating that disseminated intravascular coagulation was a key pathophysiologic feature of experimental epizootic hemorrhagic disease in WTD.[20] Later, multiple studies performed at SCWDS in BSL2 environments provided insights into the pathogenesis, vector biology, clinical signs, and immune responses of WTD infected with epizootic hemorrhagic disease virus.[23-26,89,103,104,106]

Ruder and colleagues demonstrated the vector competence and susceptibility of WTD to a nonendemic serotype of epizootic hemorrhagic disease virus (EHDV7); this work highlighted the importance of serotype-specific diagnostic tests during hemorrhagic disease outbreaks.[100,101] In 1972, Hoff and Trainer infected 3 WTD with an attenuated Trinidad vaccine strain of Venezuelan equine encephalomyelitis virus by using various routes of inoculation; studies were conducted in "tight isolation facilities at the University of Wisconsin Charmany Research Center."[40]

Throughout the past 20 y and longer, numerous studies have been performed at the SCWDS under BSL2 containment on tickborne pathogens involving WTD including *Anaplasma* spp., *Ehrlichia* spp., and *Borrelia* spp.[8-10,54,63,65,73,108,109,112,122,123] (Figure 2). Over the past 10 y, experimental infection studies with *Mycobacterium avium* subsp. *paratuberculosis*,[77] the prion agent of chronic wasting disease (CWD),[29,31,35,36,37] and bovine viral diarrhea virus[96,97] have been performed under BSL2 containment at the National Animal Disease Center (NADC) in Ames, IA.

Studies on CWD using WTD began at Colorado State University in the late 1990s. The first long-term study, which was almost 2 y in duration, was published in 2006.[57] Numerous subsequent studies using samples from the original study or similar biocontainment protocols produced seminal papers on the presence of infectious prions in saliva and blood, transmission through environmental exposure, the presence of infectious prions in B cells and platelets, and aerosol transmission of infectious prions.[12,16,17,28,30,32,38,39,55-57] Biosecurity measures included "showering in procedures, wearing of Tyvek clothing, face masks, head covers, and footwear."[57,59]

Other BSL2 studies involving WTD include those on CWD at the University of Wisconsin and the USDA's National Wildlife Research Center (Ft Collins, CO); bovine viral diarrhea at Auburn University, Purdue University, and the Sybille Conservation Education and Wildlife Research Center in Wyoming;[46,47,70,84,85] adenovirus hemorrhagic disease at the University of California in Davis;[121] La Crosse virus at the University of Wisconsin;[75] *E. coli* 0157:H7 hemorrhagic disease at SCWDS;[19] *Parelaphostrongylus tenuis* infection at the University of New Brunswick, Canada;[14,15] and *Ehrlichia chaffeensis* at Oklahoma State University (Stillwater, OK).[3,44,67,68] (Figure 2).

| Facility | Agent | Year(s) | Reference(s) |
|---|---|---|---|
| Tickborne diseases | | | |
| Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, GA | *Anaplasma odocoilei sp. nov.* | 2013 | 108 |
| | *Anaplasma* sp. | 2003 | 65 |
| Southeastern Cooperative Wildlife Disease Study | *Anaplasma phagocytophilum* | 2005 | 109 |
| Nuevo Laredo, Tamaulipas, Mexico[a] | *Anaplasma marginale* | 1971 | 53 |
| Oklahoma State University, Stillwater, Oklahoma | *Ehrlichia chaffeensis* | 2013 | 3, 44, 67, 68 |
| Southeastern Cooperative Wildlife Disease Study | *Ehrlichia chaffeensis* | 1994–2005 | 8, 10, 112 |
| Southeastern Cooperative Wildlife Disease Study | *Ehrlichia* sp. closely related to *Ehrlichia ruminatum* | 2008 | 122 |
| Southeastern Cooperative Wildlife Disease Study | *Ehrlichia ewingii* | 2002 | 123 |
| Southeastern Cooperative Wildlife Disease Study | *Borrelia lonestari* | 2006 | 63 |
| Southeastern Cooperative Wildlife Disease Study | *Borrelia burgdorferi* | 1992–1994 | 54, 73 |
| Knipling–Bushland United States Livestock Insects Research Laboratory, Kerrville, TX | *Babesia bovis* | 2015 | 110 |
| Prions | | | |
| National Animal Disease Center, Ames, IA | Chronic wasting disease | 2006–2011 | 35–37 |
| | Scrapie | 2011 | 29 |
| Prion Research Center, Colorado State University, Fort Collins, CO | Chronic wasting disease | 2006 - 2015 | 12, 16, 17, 28, 32, 38, 39, 55–57, 70 |
| Wildlife Research Center, Colorado Division of Wildlife, Ft Collins, CO | Chronic wasting disease | 2011-2013 | 45, 60, 99 |
| University of Wisconsin, Madison, WI | Chronic wasting disease | 2011 | 46, 47 |
| Bacterial diseases | | | |
| National Animal Disease Center | *Mycobacterium avium* subsp. *paratuberculosis* | 2007 | 77 |
| Southeastern Cooperative Wildlife Disease Study | *Escherichia coli* 0157:H7 | 2001 | 19 |
| Wild Animal Disease Center and Department of Pathobiology, Colorado State University | *Mycobacterium paratuberculosis* | 1983 | 117 |
| Texas A&M University, College Station, Texas | *Salmonella meleagridis* | 1970 | 98 |
| New York State Conservation Department and the New York Department of Health, Albany, NY | *Leptospira pomona* | 1962 | 93 |
| Viral diseases | | | |
| Southeastern Cooperative Wildlife Disease Study | Epizootic hemorrhagic disease | 1996 –2012 | 23-26, 88,89, 100, 101 |
| Animal and Plant Health Inspection Service Wildlife Research Facility, Colorado State University, Ft Collins, CO | Epizootic hemorrhagic disease | 2010 | |
| Ontario Veterinary College, University of Guelph, Guelph, Ontario | Epizootic hemorrhagic disease | 1971 | 20 |
| The Rockefeller Institute and State of New Jersey Department of Conservation and Economic Development, Division of Fish and Game, Trenton, NJ | Epizootic hemorrhagic disease | 1960 | 102 |
| Southeastern Cooperative Wildlife Disease Study | Bluetongue virus | 1988–1997 | 41,42, 106 |
| University of Wisconsin | Bluetongue virus | 1968 | 114 |
| National Animal Disease Center | Bovine viral diarrhea virus | 2008–2012 | 96,97 |
| College of Veterinary Medicine, Auburn University, Auburn, AL | Bovine viral diarrhea virus | 2007–2012 | 84, 85 |
| School of Veterinary Medicine, Purdue University, West Lafayette, IN | Bovine viral diarrhea virus | 2009–2012 | 69,91,92 |
| Sybille Conservation Education and Wildlife Research Unit, Wyoming Game and Fish Department, Wheatland, WY | Bovine viral diarrhea virus | 1997 | 111 |
| Veterinary Diagnostic Laboratory, School of Veterinary Medicine, University of California, Davis, CA | Adenovirus hemorrhagic disease | 2001 | 121 |
| University of Wisconsin | La Crosse virus | 1996 | 75 |
| Oklahoma Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Oklahoma State University | Malignant catarrhal fever | 1981 | 116 |
| Walter Reed Army Institute of Research, Washington, DC | Jamestown canyon virus | 1979 | 115 |
| Walter Reed Army Institute of Research | Keystone virus | 1979 | 115 |
| University of Wisconsin, Charmany Research Center | Venezuelan equine encephalomyelitis virus | 1972 | 40 |
| Other parasitic diseases | | | |
| University of New Brunswick, Fredericton, New Brunswick, Canada | *Parelaphostrongylus tenuis* | 2002–2004 | 14, 15 |
| University of Wisconsin Charmany and Reider Experimental Research Facility, University of Wisconsin, Madison, WI | *Fascioloides magna* | 1979 | 21 |
| Ontario Veterinary College, Guelph, Ontario, Canada | *Fasciola hepatica* | 1974 | 86,87 |

**Figure 2.** Experimental infection studies involving WTD in BSL1 or BSL2 biocontainment.

## Infectious Disease Research Involving in BSL3 Environments

The first published reports in peer-reviewed journals regarding the use of WTD in BSL3-type biocontainment facilities were experimental infection studies with rinderpest and peste des petits ruminants viruses[33,34] that were performed at Plum Island Animal Disease Center (PIADC) in Greenport, NY in 1975 (Figure 3). Studies on rinderpest and peste des petits ruminants viruses demonstrated the susceptibility of WTD to both viruses, highlighting the significant concern about the potential role of wildlife in the propagation of foreign animal diseases. In these studies, deer were "handled under observation in strict

| Facility | Agent | Year(s) | Reference(s) |
|---|---|---|---|
| National Animal Disease Center, Ames, IA | *Mycobacterium bovis* | 1999–2015 | 72, 77-83, 105 |
| Colorado State University, Fort Collins, CO | Northern European bluetongue virus, serotype 8 | 2013 | 13 |
| National Centre for Foreign Animal Disease, Winnipeg, Manitoba, Canada | Foot and mouth disease virus | 2012 | 62 |
| Texas A&M, College Station, Texas | *Brucella suis* | 1999 | 90 |
| Plum Island Animal Disease Center, Orient Point, NY | *Cowdria* (*Ehrlichia*) *ruminatum* | 1987 | 6 |
| Plum Island Animal Disease Center | Peste des petits ruminants virus | 1976 | 33 |
| Plum Island Animal Disease Center | Rinderpest | 1975–1976 | 34 |

**Figure 3.** Experimental infection studies involving WTD in BSL3 biocontainment.

isolation during the course of the experiment." At this time, safety procedures and equipment at the facility included "air-tight animal rooms; air-sealed (gasket) doors; and automatic wash-down airlocks."[52] The center's biosafety standard was presented to the American Biologic Safety Association for consideration in defining the 4 levels of biocontainment used for animal studies with infectious agents.[5] In 1987, studies at Plum Island demonstrated that WTD develop a rapid onset of neurologic disease and pulmonary edema after intravenous inoculation of *Cowdria ruminantium* (now termed *Ehrlichia ruminantium*)-infected blood.[6] The authors concluded that WTD "could play a major role in the spread and maintenance of this organism if it were ever introduced into the United States."

More recently, WTD have been used in BSL3 containment for extensive studies on the pathogenesis and diagnosis of *Mycobacterium bovis* infection;[72,77-83,105] pathogenesis and deer-to-cattle transmission of foot-and-mouth disease virus;[62] and pathogenesis and patterns of viremia after experimental infection with a Northern European strain of bluetongue virus (BTV8)[13] (Figure 3). These studies were performed in BSL3 or BSL3Ag high-containment facilities at NADC, National Centre for Foreign Animal Disease (Winnipeg, Manitoba, Canada), and the BSL3 Animal Disease Laboratory at Colorado State University (Fort Collins, CO), respectively.

Using WTD in modern biocontainment facilities, especially at the BSL3 or BSL3Ag levels, presents unique challenges and requires complex housing specifications as well as care and handling practices unavailable at many institutions. As described in the following paragraphs, research on *M. bovis* infection in WTD at NADC serves to illustrate many of the unique challenges posed by long-term housing of WTD under high-biocontainment conditions. All research at NADC was conducted humanely according to protocols approved by the NADC Care and Use Committee and in accordance with the *Guide for Care and Use of Laboratory Animals*[43] and the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*.[18]

## Animal Behavior Considerations

Like other ruminants, deer have a central area of binocular vision with peripheral monocular vision thus creating a very wide visual field (approximately 300°). Their depth perception, ability to detect movement, and vision under low-light conditions are excellent.[58,61] Their hearing and directional capabilities for sound detection are remarkable also.[61] As such, under almost all conditions their ability to detect humans initiates a sense of alertness and sometimes flight behavior. The raised tail of a WTD is a visual signal of danger—if one animal runs, others will follow.[61] This following behavior can be used to an animal caretaker's advantage when moving deer from one room to another.

Rooms for housing WTD are both heated and air-conditioned. Temperature is maintained between 17 and 19 °C, with relative humidity at 21% to 40%. Rooms are under negative pressure, with an airflow rate of 10 to 11 air changes hourly, and on a 12:12-h light:dark photocycle.

WTD are particularly nervous and flighty, often making sudden movements when startled. Due to this flight response, it is not uncommon for deer to run, jump, and attempt to escape when personnel enter the animal room. Consequently, gating and penning must be of sufficient height to prevent deer from escaping. One study showed WTD would jump a fence 2.1 m high but not one 2.4 m tall.[113] Still, some suggest that a height of at least 3 m should be considered.[49] When deer are startled, there is a high risk of slips or falls that can result in contusions, lacerations, or fractures. Therefore, the surface character of the flooring should reduce slipping and falling but be easily sanitized. Depending on flooring type, hooves may require frequent trimming, which generally involves manual or chemical restraint. Flooring at a biocontainment facility at Colorado State University where deer are housed is described as a mixture of sand and epoxy, which results in relatively normal hoof wear.[59]

Due to the inherent impulse of deer to flee when approached, it is useful to have a perceived 'safe' location to which deer can relocate when personnel enter the animal room. One design that has proven effective in the BSL3Ag facility at NADC is a room that contains a U-shaped animal space (Figure 4). As personnel enter one side of the room for cleaning and feeding, deer move to the other side, out of sight of the caretaker. After one half of the room is cleaned, deer are allowed to move back to the recently cleaned area, allowing cleaning of the opposite side of the room. An additional advantage to the U-shape design is that handling equipment can be placed parallel to the alleyway connecting the 2 sides of the room. When handling is required, deer can be moved to one side of the room, handled through the chute, and exited into the opposite side of the room.

As with many animal species kept in research settings, barbering—both self-directed and partner-directed—is common in WTD,[94] particularly after movement from outside facilities into containment housing. In WTD, it is unclear whether barbering is a result of boredom, anxiety, distress, crowding, social hierarchy, or a combination of factors. As is common in other species, WTD ingest the pulled hair; as such, trichobezoars in the rumen or reticulum are often found at necropsy. At NADC, enrichment devices have empirically decreased barbering such as hanging puzzle feeders containing cracked corn; the height of which is altered periodically for variety. In addition, treats in the form of peanut butter or jelly applied to hanging bucket lids are also used as enrichment.

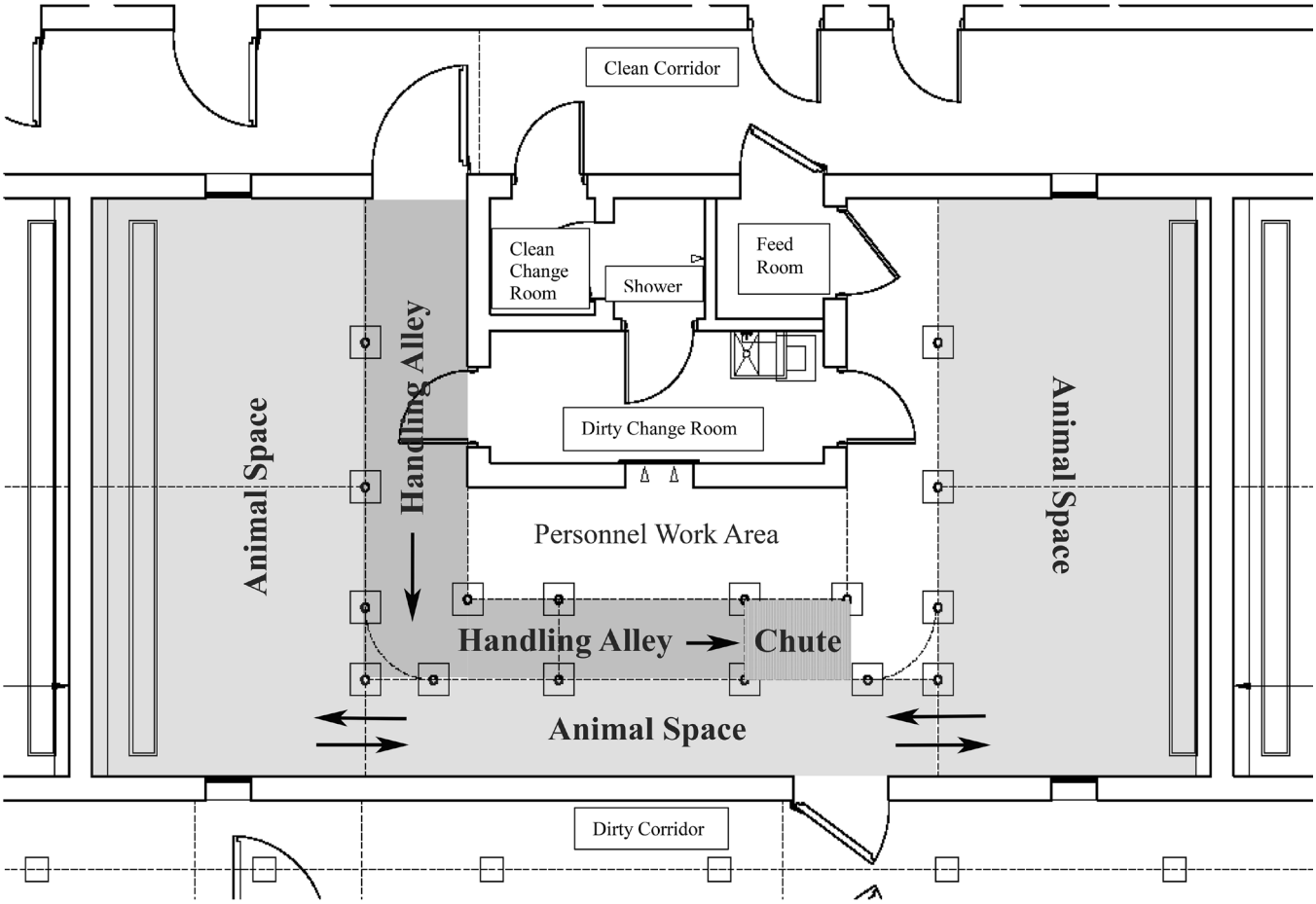


**Figure 4.** Schematic of BSL3 animal room used for housing WTD at the National Animal Disease Center (Ames, IA). As personnel enter one side of the animal space for cleaning and feeding, deer move to the opposite side, out of sight of the caretaker. After one half of the room is cleaned, deer are allowed to move back to the recently cleaned area, allowing cleaning of the opposite side. Handling equipment is placed in an alleyway, parallel to the animal space alleyway connecting the 2 sides of the room. When handling is required, deer can be moved to one side of the animal space, handled through the chute, and exited into the opposite side of the animal space.

## Penning, Gating, and Animal Handling

For use with WTD, gates, latches, feeders, and watering devices must be designed without sharp corners or angles. Restraint devices specific for handling deer are most effective. For WTD, 'drop-floor' chutes (for example, the Deerhandler [Delclayna, Alberta, Canada]) are preferable to traditional livestock squeeze chutes. Devices designed specifically for use with WTD minimize jumping yet allow considerable access to the animal for examination and treatment. Manual restraint in such a chute is suitable for blood collection, foot trimming, artificial insemination, and the administration of drugs or vaccines by oral, intramuscular, and subcutaneous routes.

Moving deer from outside facilities into containment rooms can be challenging. In addition to the potential for physical trauma, diet transition can present a considerable hurdle. At NADC, we have observed periods of anorexia that last 24 to 48 h after movement and often accompanied by temporary hematochezia, which is self-limiting. Beginning 2 wk prior to animal movement, feed is transitioned to alfalfa cubes (rather than long-stem hay) and deer pellets with 18% protein content (for example, Trophy Image Pellets [Kent Nutrition Group, Muscatine, IA]). After WTD have been moved, Hydration Hay (Purina Animal Nutrition LLC, Shoreview, MN) is added to the alfalfa cubes and deer-pellet diet to encourage eating and maintain hydration. Long-stem hay is avoided inside biocontainment housing, because of possible obstruction of drains and plumbing.

## Occupational Hazards

The safety of personnel is important when working with WTD inside biocontainment facilities. Knowledge of deer behavior, common maladies of deer, animal restraint, and appropriate training are critical for both animal and personnel safety.[95] Hand-raising fawns provides considerable time for animal caretakers to observe normal behavior and recognize signs associated with illness or aggression. The animal's tendency to flee may place personnel at risk of collisions with deer attempting escape from confinement. Veterinarians should be familiar with diseases of deer, particularly those associated with nutrition, stress, and trauma. While hand-raising fawns, veterinarians face many of the common illnesses of neonatal ruminants, such as enteritis, pneumonia, and dehydration.

In terms of decreased stress and risk of injury to both animals and personnel, the benefits of using hand-raised fawns, which have been acclimated to humans and indoor housing, cannot be overstated. Bottle-feeding and hand-raising deer fawns have a profound effect on their suitability as research subjects in general and inside biocontainment housing in particular. Hand-raising is most effective when done inside a building or space reflective of containment housing. A reliable source of fawns is

crucial, because hand-raising should begin within 24 h of birth. At NADC, we maintain a breeding herd of WTD as a source of healthy and acclimated deer with a known medical history. The exception to the advantage of hand-raised deer is the breeding buck. Hand-raised deer have a greatly reduced flight distance and will tolerate close physical associations with humans. During the mating season, an intact buck becomes highly aggressive and territorial, and an aggressive buck acclimated to humans may charge animal caretakers, creating a dangerous situation.

## Extralabel Drug Usage and Judicious Use of Antimicrobials

Farmed deer are considered a 'minor species' by the US Food and Drug Administration. According to the Minor Use Animal Drug Program, the only drugs approved for use in WTD as of January 28, 2017, are xylazine (anesthetic agent) and yohimbine (anesthesia reversal).[66] As such, the use of all other drugs is extralabel, which is defined as use in a species not listed in the labeling. Any drug should be used only under the direction of a licensed veterinarian and in the context of a valid veterinarian–client–patient relationship.

Even with good management, appropriate nutrition, appropriate parasite control, and effective vaccination strategies, there will be a need for antimicrobials to treat disease and reduce pain and suffering. Prudent use of antimicrobials will help minimize antimicrobial resistance in bacteria. The use of antimicrobials prophylactically is controversial and must be carefully examined—as should the stress and risk of injury associated with catching, restraining, and treating sick deer. The use of prophylactic antibiotics at NADC is the result of years of empirical evidence coupled with recommendations from veterinarians experienced in the health management of farmed deer herds. The vaccine regimens, parasite control, and antibiotic usage we describe here have proved successful under our conditions, but we in no way imply that alternative practices are inferior. For all products, the dosages we use are those listed on product labels for cattle.

## Breeding Herd Management

In the WTD breeding herd at NADC, we maintain a buck:doe ratio of 1:10 to 1:15. As such, our herd consists of 30 to 40 does and 2 or 3 bucks. Except during the breeding season, bucks are housed apart from does on approximately 1.5 acres of pasture, whereas the does are housed on approximately 4 acres of mixed-grass pasture. Both types of pasture contain windbreaks and feeders. Bucks are introduced into the herd for breeding in mid-November and are removed in late January. With a gestation of 195 to 205 d, fawning typically begins in early June, peaks in mid- to late June, and ends by early August.

In April and again in October, all WTD are processed through a drop-floor chute (Figure 5) for fecal and blood collection, deworming with ivermectin, and treatment with a single dose of oxytetracycline as a prophylactic measure for handling-related injuries. In addition, in April, WTD receive a cattle foot-rot vaccine (Fusogard, Elanco Animal Health, Greenfield, IN) to prevent digital dermatitis, and Bovi Sera (Colorado Serum, Denver, CO) as an aid in the prevention and treatment of enteric and respiratory conditions caused by *Trueperella pyogenes*, *E. coli*, *Mannheimia haemolytica*, *Pasteurella multocida*, and *Salmonella typhimurium*. In October, deer receive a multivalent *Clostridium* spp. toxoid (Ultrabac 8, Zoetis, Parsippany, NJ) and a combination bovine respiratory and leptospirosis vaccine (Triangle 10, Boehringer Ingelheim Vetmedica, St Joseph, MO). Also in October, for personnel safety and to decrease severe injuries resulting from interanimal aggression, antlers (in the hard-antler stage) are removed by using an obstetric wire saw. It is important to remove the antler above the pedicle to prevent pedicle damage (Figure 6). Damage to the antler pedicle can result in misshapen antlers in all subsequent seasons. Although antler velvet is highly vascular and innervated, thus requiring anesthesia and analgesia for removal, hard antler is avascular, lacks nerve supply, and is considered dead bone;[1,119,120] as such, anesthesia and analgesia are not necessary for the removal of hard antler.

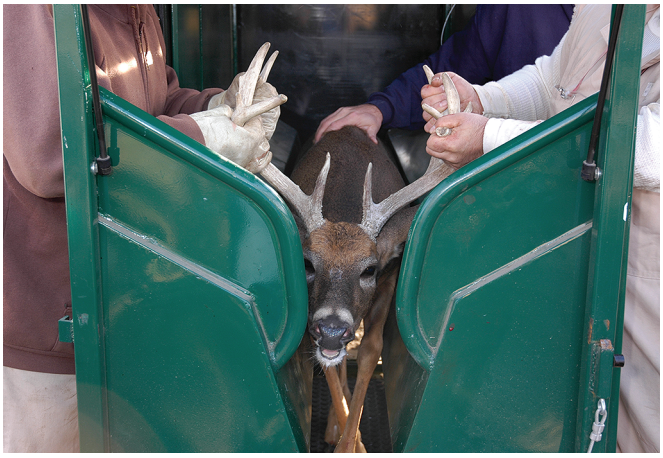

**Figure 5.** A WTD restrained in a drop-floor chute; its feet are off the ground, preventing jumping. A caretaker applies firm downward pressure over the deer's thoracic or lumbar spinal region to ensure that the animal remains restrained in the V-shaped region of the chute.

Deer are browsers or selective grazers, preferring high-quality forages. Breeding animals require 2.5% of their body weight in dry matter and 10% to 12% crude protein for maintenance. Rations for breeding deer should be 14% to 19% protein, whereas growing rations should contain 16% to 20% crude protein. Because our breeding herd often contains younger, growing animals as well as mature deer, we feed a single commercial pelleted feed containing 18% crude protein (Trophy Image Pellets, Kent Nutrition Group) year-round. The feeding rate is dependent on the quality of the hay or pasture and the body condition score and metabolic status of the animals. Trace mineral salt blocks and good-quality hay are provided unrestricted year-round; however, hay consumption varies with season and pasture condition.

## Fawn Rearing

Animal care personnel at NADC care for multiple species of livestock. Accordingly, to prevent the introduction of disease (for example, malignant catarrhal fever, paratuberculosis, cryptosporidiosis) from other animals, personnel wear clothing and footwear dedicated for use in the deer pastures. Prior to fawning, the pasture is mowed, leaving 2 strips (approximately 2 to 3 feet wide) of tall pasture grass that run the length of the pasture. These strips of pasture grass provide a place for newborn fawns to hide. Beginning in late May, personnel search the pasture twice daily for newborn fawns, which are often located in the tall strips of grass. To further decrease the potential for spreading disease when handling fawns, personnel wear exam gloves, changing them between fawns. Within 24 h of birth, all fawns are removed from the pasture and transported to a fawn-rearing facility. Medium-sized portable dog crates are

**Figure 6.** To prevent serious injury to other animals or personnel, antler is removed by using an obstetric wire saw placed above the antler pedicle to avoid damage to the pedicle. Antler is removed only in the hard-antler stage once all the velvet has been rubbed off.

used to transport fawns and are sanitized with an appropriate disinfectant (Virkon S, duPont Animal Health Solutions, Wilmington, DE) after each use.

The weight, sex, and body temperature of each fawn is recorded in an individual animal record; when known, the identity of the dam is recorded also. On admission to the fawn-rearing facility, each fawn receives a combination product containing *Clostridium perfringens* type C antitoxin and a therapeutic antibody specific to *E. coli* (Ecolizer +C20, Novartis Animal Health, Larchwood, IA), a bovine probiotic (Probios, Vets Plus, Menomonie, WI), and a single dose of florfenicol (Nuflor, Merck Animal Health, Madison, NJ). The navel is treated with 7% iodine solution. Infections at the site of ear-tag placement, sometimes resulting in severe inflammation, have been noted historically; therefore, at admission, the left ear of each fawn is punched for tag placement, which occurs 4 d later. In the intervening 4 d, the punch site is treated with antibiotic ointment (Animax Fougera Pharmaceuticals, Melville, NY). For easier identification, ear tag numbers begin with the year that the fawn is born; female tags are yellow, and male tags are white. For further identification, microchips (EZid, EZidAvid, Greeley, CO) are placed subcutaneously, either to the right of the anus or on the ventral surface of the tail.

Fawns are housed individually in pens bedded with clean straw for as long as 2 wk. Milk consumption, defecation, and urination are recorded daily. After 2 wk of age, fawns are grouped by age and sex and housed together (4 or 5 fawns per group) in pens bedded with clean oat straw. At approximately 30 d of age, fawns are allowed access to larger, outdoor pens. Soiled bedding is removed and replaced with clean fresh straw daily. Animal care personnel don barn-dedicated clothing and boots upon entering the fawn-rearing facility. Boot baths containing an appropriate disinfectant (Wexcide, Wexford Labs, Kirkwood, MO) are placed strategically at building entry and exit points and entry points to pen and crate areas.

Feeding begins with the youngest fawns. Ill fawns are fed last; signage is posted on crates or pens to alert personnel regarding the presence of an ill fawn. Fawns are fed twice daily (approximately 0700 and 1400) with warmed goat colostrum for the first 4 feedings (Figure 7), followed by a 50:50 mixture of colostrum and doe milk replacer (Zoologic Doe Milk Replacer, PetAg, Hampshire, IL), and eventually milk replacer only, with a daily intake goal of 10% to 20% of the fawn's body weight. During this dietary transition period, nutritional scours may occur and are treated, as described elsewhere,[59] with a combination of oral probiotic (Probios, Vets Plus), kaolin pectin (Kaolin–Pectin, Durvet, Blue Sprints, MO), and subcutaneous fluids. Caution should be taken to avoid overfeeding, which can increase fawn morbidity and mortality through conditions such as abomasal bloat. Milk replacer is prepared fresh for each feeding, and exam gloves are changed between fawns or groups of fawns. Unused and unconsumed milk is discarded. Bottles are washed in hot soapy water and rinsed thoroughly after each use. Beginning at 4 d of age, the fawns are offered fresh black dirt, which is changed every 3 d; fresh dirt is believed to aid establishment of a healthy intestinal microbiota and serve as a source of micronutrients.[51] To further aid the establishment of a healthy intestinal microbiota, bovine probiotic (Probios, Vets Plus) pastes or powders are added to the milk replacer daily. Fawns are offered fresh water without restriction, along with small amounts of alfalfa and a mix comprising 75% deer pellets of 18% protein content (Trophy Image Pellets, Kent Nutrition Group) and 25% cracked corn.

While feeding, fawns are stimulated to urinate and defecate by gentle rubbing of the perineal area by using an unscented baby wipe, which is then discarded. Stimulation is continued until fawns demonstrate the ability to urinate and defecate unaided (typically 3 wk of age). Urination and defecation are recorded, and the physical consistency of feces is characterized (that is, normal, paste-like, pudding-like, or watery). Coccidiosis can occur, resulting in diarrhea (which may or may not contain blood) but can be treated as in bovine calves by using amprolium (CoRid, Merial, Duluth, GA).

Treats (for example, apples) can be offered to fawns beginning at 4 wk of age. Offering treats encourages socialization with animal care personnel. Animal care staff are encouraged to spend time with the fawns to acclimate them to handling and physical examination. Weaning begins when the youngest fawn in a group reaches 60 d of age, but only when all fawns in the group are consistently eating alfalfa and pelleted feed. The duration of the weaning process is typically 2 wk but may be shorter for large, vigorous fawns.

Male fawns are surgically castrated at approximately 75 d of age. An intramuscular combination of xylazine (1 mg/kg; Rompun, Bayer, Leverskusen, Germany) and ketamine (5 mg/kg; Ketalar, Par Pharmaceutical, Spring Valley, NY) typically is used for anesthesia and is followed by subcutaneous tolazoline (4 mg/kg; Tolazine, Lloyd Laboratories, Shenandoah, IA) for reversal, but other anesthetic regimens are available.[50,71] At the time of castration, each fawn receives a dose of oxytetracycline to prevent castration-related infections. The timing of castration is important. Antler development is under hormonal control, primarily testosterone. If a fawn is castrated before the antler pedicle (the specialized generative tissue on the dorsum of the frontal bone) is formed, no generative tissue forms, and no antler develops.[22] If the pedicle has formed, castration results in a small, velvet-covered antler, which is never cast.

## Conclusions

Using WTD in any type of research setting is challenging, but it is especially difficult inside the confines of a BSL3 facility. Nevertheless, in our experience, the use of healthy, hand-raised fawns, which have a known medical background, coupled with acclimation to indoor housing, human presence, and physical handling make for superior research subjects compared with animals unaccustomed to such environments. The use of hand-raised, acclimated WTD increases confidence in research results and facilitates animal care and handling. In addition, and more

| Day | Morning feeding | | Evening feeding | |
|---|---|---|---|---|
| | Feed | Volume (mL) | Feed | Volume (mL) |
| 1 | Goat colostrum | 60 | Goat colostrum | 60 |
| 2 | Goat colostrum | 90 | Goat colostrum | 120 |
| 3 | Colostrum with doe milk replacer[a] | 120 | Colostrum with doe milk replacer | 120 |
| 4 | Colostrum with doe milk replacer | 150 | Colostrum with doe milk replacer | 180 |
| 5 | Doe milk replacer | 220–320 | Doe milk replacer | 220–320 |
| 6 | Doe milk replacer | 220–320 | Doe milk replacer | 220–320 |
| 7 | Doe milk replacer | 220–320 | Doe milk replacer | 220–320 |
| 8–30 | Doe milk replacer | 350–450 | Doe milk replacer | 350–450 |

**Figure 7.** Sample feeding schedule for WTD fawns.
[a]Zoologic Doe Milk Replacer is available from PetAg (Hampshire, IL).

importantly, hand-raising improves animal wellbeing, decreases animal stress, and avoids painful injuries.

As human populations and their livestock encroach on traditional wildlife habitat, new diseases will emerge and known diseases will arise in previously unaffected species.[7] As such, the demand to conduct infectious disease research on wildlife will increase, as will the need for facilities in which to safely conduct such research. Principles and practices that have proved effective for safe, humane research in laboratory animals and livestock will need to be applied to wildlife species. Many issues associated with the use of wildlife are common to research in all animal species, including overall animal wellbeing, enrichment to reduce stress and boredom, prevention of disease introduction, diagnosis and treatment of common maladies, appropriate nutrition, and safe and humane animal handling. In the case of WTD, there is a long history of infectious disease research conducted at ever-increasing levels of sophisticated biosecurity, demonstrating that although challenging, this type of research can be conducted, and valuable insights can be gained.

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