State of Knowledge Regarding Transmission, Spread, and Management of Chronic Wasting Disease in U.S. Captive and Free-Ranging Cervid Populations (2024)

DETAILS

209 pages | 8.5 x 11 | PAPERBACK

ISBN 978-0-309-71478-5 | DOI 10.17226/27449

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SUGGESTED CITATION

National Academies of Sciences, Engineering, and Medicine. 2024. *State of Knowledge Regarding Transmission, Spread, and Management of Chronic Wasting Disease in U.S. Captive and Free-Ranging Cervid Populations*. Washington, DC: The National Academies Press. https://doi.org/10.17226/27449.

NATIONAL ACADEMIES Sciences Engineering Medicine

NATIONAL ACADEMIES PRESS
Washington, DC

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Committee on the Review of Transmission and Geographic Spread of Chronic Wasting Disease in U.S. Cervid Populations

Board on Agriculture and Natural Resources

Board on Animal Health Sciences, Conservation, and Research

Division on Earth and Life Studies

Consensus Study Report

Prepublication Copy

NATIONAL ACADEMIES PRESS 500 Fifth Street, NW Washington, DC 20001

This activity was supported by a contract between the National Academy of Sciences and U.S. Geological Survey (Contract No. AWD-008148). Any opinions, findings, conclusions, or recommendations expressed in this publication do not necessarily reflect the views of any organization or agency that provided support for the project.

International Standard Book Number-13: 978-0-309-XXXXX-X
International Standard Book Number-10: 0-309-XXXXX-X
Digital Object Identifier: https://doi.org/10.17226/27449

This publication is available from the National Academies Press, 500 Fifth Street, NW, Keck 360, Washington, DC 20001; (800) 624-6242 or (202) 334-3313; http://www.nap.edu.

Printed in the United States of America.

Suggested citation: National Academies of Sciences, Engineering, and Medicine. 2024. *State of Knowledge Regarding Transmission, Spread, and Management of Chronic Wasting Disease in U.S. Captive and Free-Ranging Cervid Populations*. Washington, DC: The National Academies Press. https://doi.org/10.17226/27449.

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Reviewers

This Consensus Study Report was reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the National Academies of Sciences, Engineering, and Medicine in making each published report as sound as possible and to ensure that it meets the institutional standards for quality, objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process.

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Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations of this report, nor did they see the final draft before its release. The review of this report was overseen by **TERRY MCELWAIN (NAM),** Washington State University, and **MICHAEL LAIRMORE (NAM),** University of California, Davis. They were responsible for making certain that an independent examination of this report was carried out in accordance with the standards of the National Academies and that all review comments were carefully considered. Responsibility for the final content rests entirely with the authoring committee and the National Academies.

Acknowledgements

There were many helpful and dedicated people involved in developing and completing this report. The committee wishes to express their thanks and appreciation to the individuals and organizations who gave their valuable time to provide information and advice to us through their participation, sharing valuable research and practice findings, and helpful insights. The committee is also indebted to a talented and hard-working staff who contributed immeasurably to the planning and execution of meetings and especially to the production of this report. We gratefully acknowledge the efforts of our Senior Program Officer Sammantha Magsino, Program Officer Susana Rodriguez, Program Assistant Malia Brown, and to the Director for the Board on Agriculture and Natural Resource, Robin Schoen for their dedication to this project and their indispensable support and encouragement. The committee also extends its sincere thanks to the internal and external reviewers for their thoughtful and helpful feedback and invaluable insights that enabled us to improve the report

The committee wishes to recognize the sponsors from the U.S. Fish and Wildlife Service and U.S. Geological Survey of the Department of the Interior and to the Animal and Plant Health Inspection Service of the United States Department of Agriculture who supported and helped to direct the report's organization and statement of task. The committee sincerely hopes that our efforts will help guide and encourage their critical plans and programs for the future. Finally, as Chair, I am especially grateful and wish to express my deepest appreciation for the talented team who served as committee members and for their passion and determination to successfully address chronic wasting disease (CWD). Although a difficult task, these members were steadfast in their commitment, embraced teamwork and collaboration, and openly and freely shared their diverse experiences and exceptional expertise.

Preface

The physicist, Stephen Hawking, once said, “I think the next century (21st) will be the century of complexity”. His prediction has become a reality, and the term complexity also describes our world of infectious diseases today. Chronic Wasting Disease (CWD) is an example of a complex disease. It is perplexing, multifaceted and involves multiple species of the family Cervidae (i.e., the deer family). It is influenced by numerous factors and drivers, and it continues to evolve at the interface of natural and human systems.

Although first observed as a syndrome in Colorado in 1967 and first reported as a disease in 1980, CWD is likely to have been present for some time prior to this initial observation. Over the last few decades, there has been a significant increase in the number of cases being diagnosed and the disease has been found in much larger geographic areas across the U.S. CWD is both an endemic and epidemic disease and may be slowly progressing or rapidly increasing in some endemic settings. CWD represents a substantial threat to both captive and free-ranging cervids.

Considering this context, the U.S. Fish and Wildlife Service, the U.S. Geological Survey of the Department of the Interior, and the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture invited the National Academies of Science to form an expert committee and to conduct an in-depth scientific review of the state of knowledge of CWD in the United States. It also requested that this committee draw conclusions about the transmission, dissemination, and effectiveness of interventions to control the disease in both captive herds and free-ranging cervid populations. This statement of task defined the scope of this report and established its purpose. These government agencies were designated as sponsors for the report based on congressional language found in America’s Conservation Enhancement Act (ACE). The goal of the committee was to create a report that addresses these points based on a review of published and ongoing research and by engaging other experts through a series of public meetings. The report has been written to help policymakers, government officials and the lay public to better understand the many dimensions of CWD. The report will also be shared with interested congressional committees, government agencies, and members of a national CWD Task Force also designated by ACE to be formed in the near future.

The committee put together a comprehensive picture of the state of knowledge for CWD including an analysis of the pathogenesis, transmission, dissemination, epidemiology, diagnostics, drivers, control strategies, and the human dimensions describing CWD. In reviewing this content, a common and consistent finding emerged in the analyses; a large body of knowledge regarding CWD has been generated in recent decades, but there is also a lack of knowledge and multiple information gaps pertaining to many aspects of the disease. While the committee acknowledges and appreciates the productive work of researchers and practitioners, the state of knowledge is still significantly limited, and the consequence has created much uncertainty about CWD. The lack of evidenced-based information has greatly constrained our understanding of the disease and has created a barrier to both quantify its impact and measure the effectiveness of current interventions to prevent and control it. Adding to the complexity of CWD, is the need to manage and accommodate different and often conflicting views, perspectives and cultures of multiple interest groups who are affected by or are themselves affecting the status of CWD. This consensus report contains findings and offers key conclusions about the state of knowledge of CWD but it purposely does not contain specific recommendations. Instead, it provides critical results to inform officials working to control CWD and to serve as a foundation for the proposed CWD Task Force to begin its deliberations.

Even with current attempts to limit and control the disease, new cases are being identified and are being found in new geographic sites in both captive and free-ranging cervids at an alarming rate. The capacity and dedicated resources to combat CWD do not seem to be commensurate with the serious consequences of the threat. The disease, simply, is too important to ignore. The many existing critical knowledge gaps need to be filled; new interventions are badly needed; a clarity of purpose and the collaboration and coordination of activities among many interest groups are essential; and a new sense of urgency seems obvious. Although CWD is challenging especially with the current deficiencies in our state of knowledge and its inherent complexity, this reality should not prevent critical actions. Not taking CWD seriously or failure to enact effective interventions will likely make the disease more expensive to address in the future and potentially more intractable, if not impossible, to prevent or control in many areas. However, the adoption and proper implementation of existing strategies, albeit imperfect, can still limit and reduce the transmission and progression of CWD. It is the hope of the committee that this report will raise the awareness of CWD and serve as an incentive to the many interest groups associated with the health and well-being of U.S. cervids to commit to collective action and engagement, with a renewed sense of purpose and urgency.

Lonnie King, *Chair*
Committee on the Review of Transmission and Geographic
Spread of Chronic Wasting Disease in U.S. Cervid Populations

Contents

Summary[1]

Chronic wasting disease (CWD) is a concerning and complex disease first observed as a syndrome in 1967 and formally described as a spongiform encephalopathy in 1980. It is a fatal infectious prion disease that causes degeneration of the brains of some species of *Cervidae* (e.g., species of deer, elk, and moose). CWD affects both captive and free-ranging cervids and has been reported in 35 states and five Canadian provinces of North America as of August 1, 2024. The disease has shown patterns of slow epidemic growth in some areas, but tends to persist where it occurs, and can contribute to lower population growth where prevalence is sufficiently high. Misfolded prion proteins (see Box S-1) that cause CWD are shed by infected cervids via saliva, semen, urine, feces, and infected tissues. They are deposited in the environment where they can persist for years. Rates of infection in some free-ranging cervid populations exceed 45 percent and can be as high as 80 percent in captive herds.

The potential social and ecological ramifications of the increased spread and prevalence of CWD are serious, including large economic costs for agencies with management responsibilities, for industries that produce or are dependent on cervids and cervid products, and for industries that support cervid hunting and hunting-related tourism activities. The cultural or food security threats that may be felt by Native American or other communities with strong reliance on or traditions tied to cervid hunting can also be considerable. Localized impacts of CWD may differ depending on ecological and social factors.

A considerable foundation of knowledge about CWD was assembled in the first two decades after its formal description. This nascent understanding of host range, diagnostic and surveillance approaches, epidemiology, risk factors, and control strategies informed the first national response plans undertaken in the United States during the early 2000s. Research, surveillance, and management of CWD has further expanded since then. Respective state and tribal governments, federal agencies within the U.S. Department of Agriculture (USDA) and the U.S. Department of Interior, affected industries, universities, and other entities have contributed to efforts to understand and control CWD across the United States. Best management practices for free-ranging cervids were reviewed in 2018 by the Association of Fish and Wildlife Agencies (AFWA), which also identified unanswered questions regarding, for example, the precise mechanisms of transmission, evolution of different prion strains, effects of population density on transmission, transmissibility to other species, and the effects of human factors on the disease spread.

In 2020, Congress passed America's Conservation Enhancement (ACE) Act (P.L. 116-188) which directs the USFWS to lead a CWD Task Force to "leverage the collective resources of Federal, State, and local agencies, [Tribal communities], and foreign governments, and resources from private, nongovernmental entities" and develop an action plan for addressing CWD in the United States. The ACE Act encouraged the Secretaries of the Interior and Agriculture to commission the National Academies of Sciences, Engineering, and Medicine (the National Academies) to produce "a special resource study to identify the predominant pathways and mechanisms of the transmission of chronic wasting disease in free-ranging and captive populations of cervids in the United States." This report summarizes the state of knowledge related to CWD that can aid the task force when prioritizing research and developing future CWD management strategies.

THE STUDY CHARGE

In collaboration with the USDA and the USFWS, the USGS requested the National Academies to convene a committee to draw conclusions about the state of knowledge regarding CWD. An ad hoc committee of 11 experts conducted a retrospective examination of published and ongoing research

[1] This summary does not include references. Citations for the information presented herein are provided in the main text.

regarding infectious doses and concentrations, modes of transmission, the means of geographic spread, the effectiveness of interventions to reduce transmission or spread, and the human societal implications of CWD. This report synthesizes that committee's efforts. The audience for this report includes the study sponsors and state, provincial, tribal, and federal agencies with interest in or responsibility for managing free-ranging or captive cervids; conservation and hunting groups or individuals; the captive cervid industry; other individuals and groups interested in CWD and cervid management; members of Congress; and the public at large.

BOX S.1
What are Prions and Prion Diseases?

Prion proteins are normally found in the body. Misfolded versions of the prion protein can lead to prion diseases. Prion diseases are neurodegenerative diseases that include CWD, bovine spongiform encephalopathy in cattle, sheep scrapie, and Creutzfeldt-Jakob disease in humans. Prion diseases affect brain and other neural tissues and functions in infected animals and are fatal. Infections occur when misfolded prion proteins induce the misfolding of normal prion proteins in a susceptible animal.

The report attempts to identify the levels of uncertainty of CWD-related knowledge and management practices. The study charge did not request recommendations for future actions; the committee provided conclusions based on existing evidence. The intent is to aid future decision makers in the prioritization of research and the further development of CWD management and mitigation strategies with the understanding that the complete control or elimination of CWD at the national or local scales may not be within reach.

DISEASE DESCRIPTION

The process by which CWD prion infection leads to disease (i.e., pathogenesis) largely parallels what is seen with other prion diseases (see Box S.1), but its modes of transmission and management are different. Oral-nasal contact with CWD prions is thought to be the most common mechanism of the transmission of CWD prion. Following natural exposure and infection, the disease progresses for more than a year. Incubation periods differ depending on the prion strain and dose, the route of exposure, the animal species involved, and, importantly, the genotype (e.g., the genetic makeup of the cervids). Infected cervids may show little to no outward signs of disease for most of the disease's incubation period, but late-stage infected cervids show signs such as changes in behavior and progressive deterioration of body condition that is expected to lead to the death of the infected animal.

A gene with a primary role in CWD infection, transmission, and pathogenesis encodes the normal prion protein *(PRNP*). This gene varies within cervid species and between cervids, cattle, and sheep. Gene variation results in differences in disease susceptibility and in what is referred to as the "species barrier" (e.g., that which prevents the crossing of the disease from one species to another). No cervid *PRNP* genotype identified, to date, is completely resistant to infection. Although prion disease susceptibility depends on a functional prion protein, the *PRNP* gene is likely not the only gene involved in disease pathogenesis.

Laboratory studies have identified multiple strains of CWD prions. The pathogenesis of the more common CWD prion strains has been well studied. Disease resulting from different strains of CWD prions in the United States are difficult to differentiate based on clinical patterns and pathology. Current techniques for prion strain evaluation do not allow a detailed analysis of the abnormal protein structure, and there is insufficient data about specific host species, their *PRNP* genetics, and their locations to allow comparison of data from different studies.

EVALUATION OF THE GENERAL STATE OF CWD KNOWLEDGE

Science, collaborative research, and experience have created a substantial body of knowledge regarding CWD in free-ranging and captive cervids. However, some knowledge is unpublished and therefore implicit. Knowledge that exists but is not publicly available may be based on prior or ongoing research or on anecdotal information. Decisions and policies informed by this knowledge could be correct but may also be fraught with uncertainty. The strategic efficacy and cost-effectiveness of those decisions may not be optimal. Furthermore, CWD-related policies may not be fully understood by the public because of a lack of access to supporting information. This could result in CWD control activities being implemented inconsistently or without persistence. More freely available data and information could resolve many uncertainties about CWD. Examination of existing data could resolve many open questions but some scientific knowledge gaps related to CWD will require new information gained through targeted laboratory and field studies and research.

TRANSMISSION AMONG CERVIDS AND FROM THE ENVIRONMENT

Conclusion 1: Multiple drivers and epidemiological factors affect CWD transmission and infectivity. The precise roles, interrelationships, and relative importance of these factors are not fully understood, cannot be fully quantified, and may differ for captive and free-ranging cervid populations.

Given the difficulty of studying CWD in natural settings, much knowledge regarding CWD derives from controlled experimental studies. However, data derived from controlled investigations—including data related to transmission—may have limited application to analogous processes occurring in nature. For example, the infectious dose, repeated exposure, persistence of CWD prions in the environment, duration of incubation and pathogen shedding by the host, the molecular makeup of the prion itself, and host genetic differences all influence transmission in laboratory settings. These are difficult to study in natural settings. The effects of numerous anthropogenic factors that likely contribute to CWD transmission and geographic spread also have not been quantified.

TRANSMISSION TO NON-CERVID SPECIES INCLUDING HUMANS

Conclusion 2: As of this writing, no cases of CWD transmission to humans have been diagnosed or reported, nor has natural transmission to non-cervid animal species been detected.

As the prevalence and geographic distribution of CWD increases, so does the risk of exposure to other potentially susceptible species. However, cases of natural CWD infection of species outside the cervid family have not been reported. CWD prions have been experimentally transmitted to species such as pigs, raccoons, ferrets, cattle, sheep, and various rodents. Most of those transmissions were achieved via injection directly into the brain rather than natural exposure routes. CWD prions can be found in fecal material of free-ranging carnivores and scavengers such as cougars, coyotes, and crows, but are thought to pass through their digestive systems without infecting them.

The potential for transmission of CWD to non-cervid species (including humans) by more natural routes of exposure (e.g., ingestion) is unknown. There is concern among some observers that communities that harvest wildlife and plants from areas of heavy cervid-use or employ traditional medicinal or ceremonial practices may experience disproportionate or unique exposures to the CWD prion that may warrant some investigation. The collective results of research to date using a variety of molecular and animal models suggest the species barrier between humans and CWD prions is likely high, though perhaps not absolute. The zoonotic potential of each CWD strain needs to be assessed. Some laboratory studies incorporating experimental amplification assays and surrogate models of human susceptibility (e.g., some species of non-human primates and transgenic mice expressing human prion proteins) have

suggested that certain strains of CWD prions may have some potential to induce the human prion protein to misfold. Those data need to be interpreted with caution as low levels of prions—perhaps below the level of clinical relevance—were amplified. The route of inoculation (e.g., oral versus direct injection into the brain) and strains being studied may account for some of the observed experimental variability.

SPREAD OF CWD

Conclusion 3: The known geographic distribution of CWD is expanding. However, the distribution is incompletely understood and likely underestimated. Inconsistent surveillance has compromised knowledge about changes in CWD distribution over time in the United States.

Organized CWD surveillance was minimal prior to 1997, but had been undertaken in several states and Canadian provinces by 2000, and has been practiced to varying degrees across the United States since 2002. The details of recent and historical surveillance efforts are unevenly available to the scientific community and the public. Despite that, and despite the difficulty detecting focal CWD outbreaks and measuring real-time spread, ample evidence suggests that the number of cases and geographic distribution of CWD have increased. Timelines of geographic spread, however, cannot be inferred reliably based only on the chronology of first detections and are likely underestimated due to inconsistently applied surveillance practices. Because management of CWD is more challenging when the disease is widespread, understanding epidemic expansion and timelines is important.

CWD surveillance is resource-intensive, costly, and unevenly implemented in many regions, but there are effective, sustainable, and coordinated surveillance strategies applicable to both captive and free-ranging cervids despite the unique challenges presented by each. Information gained through surveillance is necessary to improve scientific understanding of the epidemiology and geospatial characteristics of CWD.

Conclusion 4: Natural movement of infected cervids and other epidemiological factors are responsible for the local distribution of CWD. Human-mediated movement of infected cervids (i.e., transport of live, dead, captive, or free-ranging cervids) and infected cervid products for commerce, recreation, conservation, and other purposes increases the likelihood of CWD spread to new geographic areas in unpredictable ways.

Multiple natural mechanisms (e.g., natural movement of infected cervids among and between herds, shedding of prions by infected cervids into the environment, and spread of CWD prions by predators and scavengers) are known or suspected to be responsible for CWD spread within a geographic region. Human activities and behaviors likely have contributed to rapid and less predictable spread of CWD across longer distances than might be expected to occur naturally. For example, transport of infected live cervids is known to spread the disease across state and international lines and to new areas far from where CWD had been previously detected, and the transport of infected dead cervids or of infected cervid parts or products (e.g., meat, velvet, and urine products)[2] is suspected but not yet demonstrated to have had a similar role in some cases. On the local scale, it is conceivable that behaviors such as improper disposal of carcass parts[3] and the use of attractants or bait to encourage congregation of cervids could increase the spread of the disease, but these have not been documented. While there are numerous state, provincial, and tribal bans and carcass import regulations prohibiting the movement of select portions of hunter-harvested cervids across jurisdictions,[4] regulatory compliance is not well studied.

[2] Discussion of cervid parts or products are intended to describe their suggested but as yet undemonstrated roles in contributing to the spread of CWD to cervid populations. This text does not describe the potential risks to humans of contact through consumption of cervid meat or through environmental contact.

[3] For example, in a manner that could contaminate the environment or be a source of infection to cervids.

[4] See https://cwd-info.org/state-and-province-carcass-import-regulations/ (accessed June 22, 2024).

How attractants, feed and feeding sites, carcasses, farm equipment, captive cervid pens, and across-fence contacts contribute to CWD transmission is being studied.

DIAGNOSTIC METHODS

Conclusion 5: Official USDA postmortem CWD diagnostic approaches are useful for disease surveillance in both free ranging and captive cervids. Newer, as yet unapproved, detection approaches may have more wide-ranging applications, including live-animal testing and screening of cervid byproducts, environmental surfaces, and other relevant materials.

Screening and diagnostic methods used to surveil potential environmental contamination and to monitor for changes and potential emergence of new strains are needed as adjuncts to established diagnostics to confront management challenges where CWD occurs. Since the early 2000s, CWD detection and surveillance has relied on standardized immunohistochemistry (IHC) or enzyme-linked immunosorbent assay (ELISA) protocols for postmortem detection of the misfolded prion protein in lymphoid and neural tissues. These are the only tests officially recognized by the USDA, and they may only be conducted by approved diagnostic laboratories. They are not widely applied to live animal testing and cannot be used for screening select biological and environmental samples that may have important roles in CWD transmission (e.g., soil, plants, insects, or bodily waste). There is a need for rapid, inexpensive, field-deployable tests useful for early CWD detection in live cervids and for environmental surveillance.

Amplification assays for detecting CWD prions have been developed (e.g., the protein misfolding cyclic amplification [PMCA] assay and the real time quaking-induced conversion [RT-QuIC] assay), but none are USDA-approved. When fully evaluated, these tests may be shown to be faster, more versatile, and more sensitive than their approved counterparts. They have proven useful for screening a range of matrices including tissue biopsies, bodily fluids, and environmental samples that are unsuitable for IHC or ELISA screening, and they have the potential to accommodate high testing volumes. However, because of variability in testing results, these amplification assays require standardization and continued validation before being considered for approval. A better understanding of the relationship between detection and infectious dose is needed.

CONTROL

Conclusion 6: Well-founded epidemiological principles inform strategies for CWD prevention or control in both captive and free-ranging cervids, beginning with effective early ongoing surveillance and followed by timely aggressive sustained local response upon the presence of CWD being discovered. Although imperfect, methods based on those principles can reduce or prevent large increases in prevalence and slow the spread of CWD when properly applied.

CWD is transmitted both directly (i.e., animal to animal) and indirectly (from the environment). As such it cannot be controlled through a single intervention as have other prion diseases (e.g., through managing feed, as for bovine spongiform encephalopathy). Current and collective knowledge regarding CWD and its control, albeit incomplete, is based on sound epidemiological principles demonstrated to be effective in the management of both animal and human infectious diseases. That knowledge is sufficient to inform interim comprehensive control strategies to slow the occurrence and spread of the disease at the local and national levels. For example, the knowledge that infected hosts, their residual secretions and excretions, and their carcasses are potential sources of infectivity can be used to inform control measures such as implementation of risk-based and targeted culling, appropriate carcass handling, compulsory surveillance, and regulation of commercial movement of cervids and products.

Experience gained from the management of other infectious diseases indicate that early ongoing surveillance and aggressive and sustained local response once CWD is detected can be effective at

preventing and controlling CWD in both captive and free-ranging cervids. Other practices based on sound epidemiological principles include:

- Situational awareness of CWD prevalence, host and environmental factors, and animal movements based on risk assessments;
- Reduction or elimination of human-facilitated spread of infected cervids or infected cervid products;
- Identification, elimination, or reduction of potential sources of indirect exposures;
- Development of realistic and plausible control goals incorporating socioeconomic, political, and cultural dimensions in a collaborative and adaptive framework; and
- Preemptive public messaging and education on CWD and prospects for control.

Infection of captive cervid herds can be limited with strong biosecurity and preventive management, for example through certifying animal sources and applying quarantine principles to infected herds. However, these cervids are still at risk, particularly in areas where CWD occurs among free-ranging cervids. The USDA Animal and Plant Health Inspection Service CWD Herd Certification Program (HCP) is a voluntary, state-administered program intended to prevent and reduce CWD transmission and spread in captive cervid herds. The HCP has contributed to limiting interstate spread of CWD; however, the total number of enrolled herds has decreased in recent years. Detection of CWD in HCP-certified herds suggests that current biosecurity measures need to be reconsidered and that indirect transmission within and among herds needs further investigation. HCP participation, alone, is not sufficient to control CWD transmission and spread. In-depth epidemiological analyses of CWD-infected herds would result in better understanding of risk factors and inform improvements in disease prevention and biosecurity.

Beyond the general CWD control measures described above, and based on accepted epidemiological practices, CWD control measures expected to be effective in managing free-ranging populations and controlling CWD prevalence include use of targeted local culling or harvest to reduce host abundance and sources of infection, and regulating or banning baiting and feeding.

Measuring the effectiveness of CWD-control strategies for free-ranging herds is challenging. Underestimating the scope and scale of the disease, constraints on design and implementation of control programs, and a lack of sustained support among interested and affected parties have affected outcomes in most situations. Knowledge gaps may result in management strategies with high levels of uncertainty. Thorough epidemiologic analyses of newly detected outbreaks are warranted to improve understanding of risk factors and improve disease prevention and biosecurity measures. Collaborative efforts to evaluate and understand the collective cross-jurisdictional portfolio of evidence and to convert existing but inaccessible or anecdotal information into accessible and actionable data will enhance the collective ability to blunt the effects of the disease in the short-term and inform adaptive management decisions. Ongoing research may then inform longer-term control solutions.

Conclusion 7: Differing philosophies and approaches to CWD management adopted by agricultural and wildlife management authorities at different levels of government impact the effective control of CWD in the United States.

A variety of local, state, tribal, and federal entities operating under different authorities, jurisdictions, regulations, guidelines, social pressures, and management strategies all have different responsibilities and approaches for managing CWD—often with inadequate resources. The result is a patchwork of non-standardized prevention and control strategies that are unevenly and inconsistently adopted, implemented, and evaluated.

Government agricultural agencies are largely responsible for managing captive cervids (generally regulated as livestock) for the economic benefit of producers and to meet market demand for cervids and

cervid products. Control efforts are focused largely on protecting captive cervids from infection, or on preventing the spread of CWD among captive cervid facilities. They may reduce the spread of disease from captive to free-ranging cervid populations, but they cannot address the spread of disease once transmitted into free-ranging populations.

In contrast, state, tribal, and federal wildlife management agencies often focus on maximizing recreational, economic, ecological, and societal values associated with free-ranging cervids. These goals may conflict with recognized successful CWD-reduction approaches. For example, culling appears to be an effective practice for controlling the prevalence of the disease in some situations, but this practice may conflict with the public preference for seeing greater numbers of cervids in the wild or to use hunting as a control tool. Tribal agencies may have the additional challenges of balancing cultural and traditional values, food sovereignty, and a subsistence economy with wildlife management priorities, while also having limited agency capacity and high administrative burden in acquiring federal funding and grant management.

Improved disease management is more likely with coordinated, complementary, collaborative, and sustained control strategies among management agencies with responsibilities for both captive and free-ranging cervids. These strategies will be based on improved understanding of the underlying genetics of reduced CWD susceptibility, and the development of and access to improved diagnostic approaches, as described earlier. Data sharing and comprehensive data analyses of CWD outbreaks are warranted. Adaptive CWD management strategies that can be customized and modified for various jurisdictions and situations have substantial advantages and merit.

> *Conclusion 8: Prevention is key to controlling the spread of CWD given that existing tools and technologies make eradication of CWD in captive or free-ranging cervid populations, once established, improbable. Ongoing and effective surveillance programs can facilitate early detection and response.*

CWD surveillance is not equivalent to management, but it is essential for informing improved prevention and control programs. Timely detection will be more likely with ongoing and effective surveillance programs. Effective and less resource intensive rapid response is more likely with early timeline detection of CWD. Experience controlling infectious disease indicates that an aggressive response to CWD when detected in a new geographic region early in an outbreak can result in the local elimination of the disease in at least some circumstances (although future re-emergence of the disease is possible). If CWD becomes established among local free-ranging cervids or in a captive herd, effective management and control strategies may keep CWD prevalence at low levels and limit the buildup and overall contribution of environmental contamination to ongoing transmission. However, it is yet unclear how long low prevalence levels can be maintained. Presently, there are no environmental treatments or habitat modifications that effectively reduce or eliminate prions in the environment. Quickly removing infected captive cervid herds from the landscape is likely important in controlling disease spread where CWD is not already established in the surrounding area.

> *Conclusion 9: Genetic selection, vaccines, environmental decontamination, and therapeutic options are being investigated as tools for CWD control, but need further inquiry and review. Although none of these approaches can, at present, replace existing forms of management and control, in the future they may, in combination with current methods, reduce CWD on the landscape.*

Current strategies focusing on active surveillance and regulations covering the movement of cervids and their byproducts are imperfect but effective. Targeted research to develop and expand new tools and strategies such as genetic selection, vaccines, environmental decontamination, and therapeutic options to prevent or control CWD are essential and deserve further study. However:

- Selective breeding of animals less susceptible to CWD as a management tool in captive cervid herds may be constrained because of the limited understanding of the different rates and lengths of exposure in herds and exposure to different CWD prion strains. Managing CWD in free-ranging populations through selective breeding is not feasible. Longitudinal studies in both captive and free-ranging populations would facilitate a better understanding of genetics on relative susceptibility to prion infection and prion shedding.
- Vaccine development efforts are in early stages of development and have yielded mixed results in rodent models and have thus far been largely unsuccessful in cervid hosts. The same is true for other potential therapeutic options. Without better knowledge of cervid and prion biology and cervid immunity against different CWD strains, vaccines and other therapies are not ready for use. Development of remote delivery systems (e.g., oral bait) may facilitate reductions in disease prevalence, at least in captive cervids.
- Environmental decontamination protocols to date include the removal of topsoil and treatment of landscapes with hydroxide, treatment of landscapes with humic acid, and exposure to high temperatures. These treatments have yet to be demonstrated as effective and ecologically responsible. Implementation of decontamination protocols may be difficult and impractical in many cases at scale. Heterogeneities in soil types, vegetation, and weather over many acres may alter the efficacy of a protocol.
- Limited laboratory evidence suggests that the impacts of CWD or its infectivity can be reduced in biological samples through therapeutic options, but there is no evidence regarding their use in live cervids or the environment.

SOCIAL AND JURISDICTIONAL FACTORS

Conclusion 10: Human behaviors can influence the transmission, spread, and consequences of CWD. Interest groups hold diverse viewpoints regarding the seriousness of CWD and about its spread, prevention, and control; their decisions may not always be informed or influenced by the best available science.

Complex human dimensions can be as influential as biological factors in the transmission, spread, and management of CWD. Lack of understanding of the social, economic, and cultural human dimensions with respect to CWD is a significant barrier to CWD management. Interested and affected parties hold different views, values, beliefs, and economic interests, and their behaviors and decision-making differ greatly. Division among them can result in a lack of participation, compliance, and collective support for CWD actions and programs. The lack of communication and coordination mechanisms related to data collection and information sharing across jurisdictions precludes an evaluation of control measures for their efficacy.

The integration of multiple disciplines and expertise—including sociology, economics, behavioral science, anthropology, and others—is needed to create innovations and strategies to better leverage human dimensions in CWD management. Translation of CWD science into decision making requires a fuller understanding of CWD gained through collaborative and coordinated open data-sharing across disciplines, organizations, and governmental entities. Such collaboration will be critical to mitigate the negative impacts of the disease on cervid population levels, minimize socioeconomic impacts of the disease, reduce potential misinformation, and to evaluate and launch effective, sustainable interventions.

ECONOMIC ANALYSES

Conclusion 11: Existing data gaps make CWD-related economic measurements and analyses difficult to quantify. These deficiencies can result in a lack of appreciation of the full impact of the

disease and in the inability to evaluate and compare the direct and indirect costs and benefits of various management strategies.

CWD is becoming more costly and consequential to manage as a result of the increasing number of cervids affected and their geographic distribution. The lack of accurate and current economic information may result in some entities and individuals undervaluing the impacts of CWD and may diminish a sense of urgency to act on CWD. Expenses associated with CWD management represent economic vulnerabilities for the federal, tribal, state, and local entities with CWD-related authorities and responsibilities. Tribal wildlife management entities are further constrained by the lack of resources, staff, and levels of administrative burden associated with the complicated processes for transfer of federal funding and grant management. Agencies may be unequipped or underequipped to meet the current challenges of CWD, and current resources may be insufficient to address CWD as it continues to spread.

Estimates of opportunity cost functions allow the prediction of future consequences of additional captive or free-ranging herds becoming infected with CWD. However, although some data regarding state and federal costs and expenditures are available, the full economic burden or impact of CWD at any jurisdictional level, cannot be quantified. At present, most jurisdictions can produce only rough estimates of the numbers and locations of free-ranging cervids and herds. Costs and benefits of CWD management options will depend on multiple variables including the current and predicted populations of both captive and free-ranging cervids, local economies and markets, and a range of human dimensions. Analyses of captive and free-ranging population levels in a wide variety of locales, and in concert with robust CWD surveillance programs, would support cost-benefit analyses of localized CWD management activities.

MOVING FORWARD WITH A COORDINATED RESPONSE

Limited knowledge related to many critical aspects of CWD, its epidemiological and biological complexity, and the diversity and competing views of various interested and affected parties make CWD challenging to address and control. However, the ability to adopt scientifically supported strategies can result in a productive, cost-effective set of interventions capable of slowing the transmission and spread of the disease and its impacts. Science, collaborative research, and experience continue to provide new knowledge and understanding of CWD in free-ranging and captive cervids. Further exploration of how to apply this knowledge in a collaborative, coordinated, and sustained manner across agencies with management responsibilities, particularly regarding the management of free-ranging populations, would improve captive and wild cervid management decisions aimed at altering the trajectory of the rate of change of CWD.

1
Introduction

Chronic wasting disease (CWD; Williams and Young, 1980) is a fatal prion disease affecting the central nervous systems of multiple cervid species (i.e., species in the "deer family"), including free-ranging and captive mule deer, white-tailed deer, elk (also known as wapiti), moose, caribou (sometimes called reindeer), red deer, and sika deer (cases in sika deer have thus far only reported in South Korea). Box 1.1 describes what prions and prion diseases are. A syndrome now understood to be CWD was first observed affecting captive mule deer in Colorado in 1967 (Williams and Young, 1980; Williams and Young, 1992). As of August 1, 2024, CWD cases have been reported in 35 U.S. states and five Canadian provinces (see Figure 1.1). CWD has independently emerged among cervids (moose, red deer and reindeer) in the European countries of Sweden, Norway, and Finland (EFSA Panel on Biological Hazards, 2019; Sola et al., 2023). It was introduced into South Korea via trade of live cervids from Canada (Sohn et al. 2002; Kim et al. 2005).

BOX 1.1
Prions, Prion Diseases, and CWD

Proteins are molecules found within the body made up of strings of amino acids that fold themselves in various ways to support a variety of cellular functions. One of these proteins is known as cellular prion protein (PrP^{C}) and is found throughout the body, with the largest concentrations occurring in the brain. Its exact function is not known. Sometimes these prion proteins misfold and form abnormal structures which cease to function properly and gain toxic properties. Abnormal prion proteins in cervids that lead to CWD are called PrP^{CWD}. These abnormal prion proteins are resistant to enzymatic breakdown, accumulate in the brain and result in an incurable and fatal prion disease called chronic wasting disease (CWD) (Prusiner, 1991; Williams and Miller, 2002). CWD is part of a group of prion diseases affecting animals and humans that are sometimes termed transmissible spongiform encephalopathies (TSEs). In a TSE, microscopic holes form in the brain giving it a spongelike appearance and affecting brain function and death as the disease advances. CWD affects cervids, but others affect sheep (i.e., scrapie) and cattle (i.e., bovine spongiform encephalopathy, BSE—sometimes called "mad cow disease".)

It is believed that a clinical syndrome observed in 1967 within a captive herd of mule deer (*Odocoileus hemionus*) at a research facility in Fort Collins, Colorado likely represents the first documented occurrence of CWD (Williams and Young, 1980). In the 12 years that followed, additional cases of the disease were observed in mule deer, Rocky Mountain elk (*Cervus canadensis nelsoni*), and black-tailed deer (*Odocoileus hemionus columbianus*) held at this and other wildlife research facilities in northern Colorado and southeast Wyoming, and in a Canadian zoo (Miller et al., 2000; Williams and Young 1980; Williams and Young 1992). It wasn't until 1978, however, that the disease was classified definitively as a spongiform encephalopathy based on the presence of "characteristic histological lesions" in the brain and spinal cord of affected animals (Kahn et al., 2004; Williams and Young, 1980; Williams and Young, 1992).

CWD prions are thought to be transmitted both directly and indirectly between cervids, and have been shown to persist in the environment for long periods (in a review by Osterholm et al., 2019; Miller and Wolfe, 2023). Cervids infected with CWD have been observed to have measurably shortener lifespans compared to uninfected compatriots (e.g., Miller et al., 2008; Monello et al., 2014; Edmunds et al., 2016; Devivo et al., 2017; Haley et al., 2020a; Ballard, personal communication to NASEM, 2023).

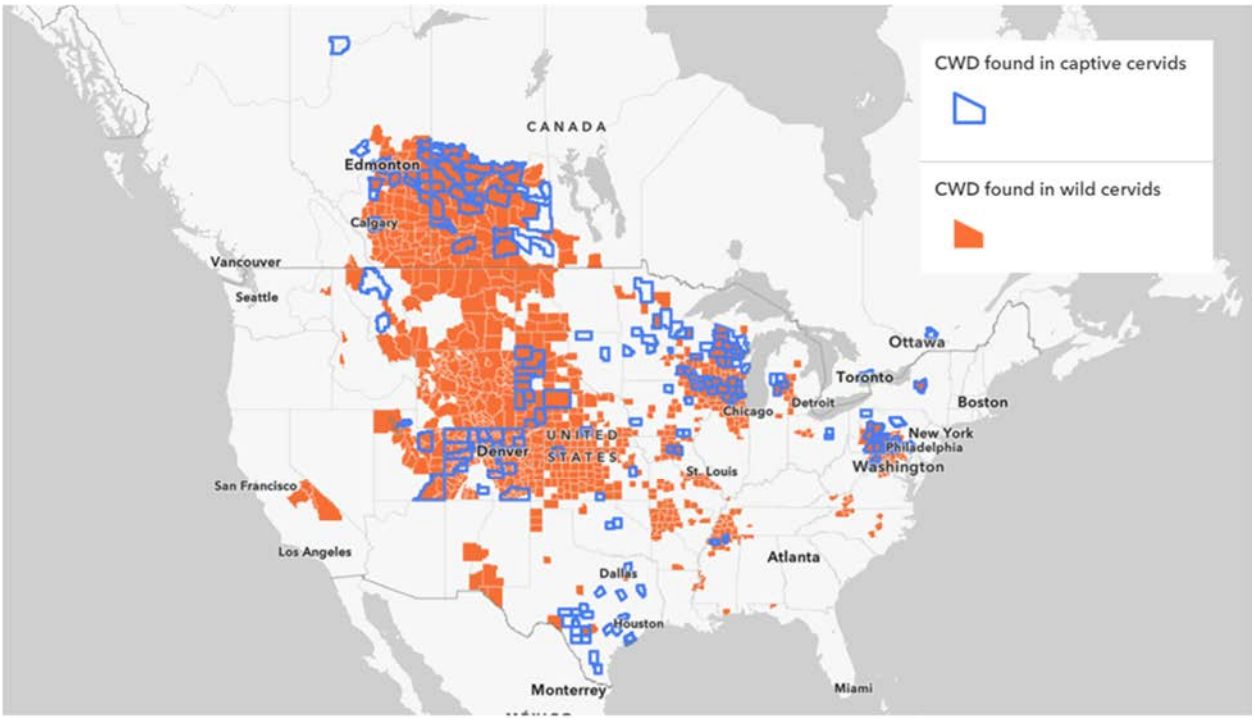


FIGURE 1.1 Known geographic distribution of chronic wasting disease (CWD) in the continental United States and Canada as publicly reported by respective state and provincial authorities as of August 2024. The colored polygons represent administrative boundaries (e.g., counties or wildlife management units) where one or more CWD case(s) has been diagnosed in captive (outlined in blue) or free-ranging (solid orange) cervid hosts since 1974. Dual coloration reflects locations with reported occurrences in both captivity and in the wild. No cases have been reported in Alaska, Hawaii, the northern Canadian provinces, or portions of Mexico not shown on the map.
SOURCE: Chronic Wasting Disease Alliance; https://cwd-info.org/map-chronic-wasting-disease-in-north-america/ (accessed August 16, 2024).

Rates of infection tend to increase over time (see Box 5.1 in Chapter 5), eventually exceeding 20 percent among adult females and twice that among adult males in some wild deer populations (e.g., Miller et al., 2008; Edmunds et al., 2016; Devivo et al., 2017; Ballard, personal communication to NASEM, 2023 Fisher et al., 2022; Saskatchewan Ministry of Environment 2024;[1] Wisconsin Department of Natural Resources[2]). Prevalence as high as 80 percent has been reported in captive herds (Keane et al., 2008a). In addition to increasing numbers of infected animals, outbreaks in some states have shown marked geographic expansion over time such that, in the most extreme examples, cases have been recorded across virtually entire states (see Figure 1.1). The emergence of CWD can become a significant mortality factor in affected cervid populations when a sufficiently high proportion of adult females become infected (Devivo et al., 2017; Edmunds et al., 2016; Ballard, personal communication to NASEM 2023; Miller et al., 2008; Haley et al., 2020a; Fisher et al., 2022; Gilbertson et al., 2022; Monello et al., 2014).

A considerable foundation of knowledge about CWD was assembled in the first two decades after its formal description by Williams and Young (1980, 1982). This nascent understanding of host range, diagnostic and surveillance approaches, epidemiology, risk factors, and control strategies (see reviews by Williams and Young, 1992; Williams and Miller, 2002, 2003; Williams et al., 2002) informed the first national response plans undertaken in the United States during the early 2000s (Chronic Wasting Disease Task Force, 2002; National CWD Plan Implementation Committee, 2002; Gillin, Mawdsley, and eds., 2018). Research, surveillance, and management of CWD intensified after the disease was declared a national emergency in the United States (Geist et al., 2017) and has become a major focus of research activity within the U.S. Geological Survey (USGS). The U.S. Department of Agriculture (USDA) also

provides research through the Agricultural Research Service (ARS) and management guidance to the captive cervid industry through the Animal Plant Health Inspection Service, Veterinary Services (APHIS-VS). The Association of Fish and Wildlife Agencies' technical report (Gillin, Mawdsley, and eds., 2018) provides a detailed technical literature review that describes best management practices and points to the many CWD-related questions that remain unanswered. Those questions regard topics such as the precise mechanisms of transmission, the evolution of different prion variants (strains), the effects of animal density on transmission, the transmissibility of CWD to other species, the effects of human factors on the disease spread, and many others.

In 2020, Congress passed America's Conservation Enhancement Act (P.L. 116-188) which directs the U.S. Fish and Wildlife Service (USFWS) to lead a CWD Task Force to "leverage the collective resources of Federal, State, and local agencies, [Tribal communities], and foreign governments, and resources from private, nongovernmental entities" and develop an action plan for addressing CWD in the nation. The ACE Act encouraged the Secretaries of the Interior and USDA to commission from the National Academies of Sciences, Engineering, and Medicine (the National Academies) "a special resource study to identify the predominant pathways and mechanisms of the transmission of chronic wasting disease in wild, captive, and farmed populations of cervids in the United States."

CHARGE TO THE COMMITTEE

In collaboration with the USDA and USFWS, the USGS requested that the National Academies convene an ad hoc multidisciplinary committee of experts to draw conclusions about the state of knowledge on CWD. Box 1.2 is the statement of task provided to the committee. The committee was to examine published and ongoing research regarding infectious doses, modes of transmission, the means of geographic spread, the effectiveness of interventions to reduce transmission or spread, and implications on human society of CWD.

BOX 1.2
Statement of Task

An ad hoc committee of experts appointed by the National Academies of Sciences, Engineering, and Medicine will review the state of knowledge about modes of transmission and means of geographic spread of chronic wasting disease (CWD) among free-ranging[a] and captive[b] populations of cervids in the United States. Specifically, the committee will draw conclusions about the state of knowledge regarding:

- The infectious dose of CWD and different modes of disease transmission among cervids;
- The means of geographic spread through cervid dispersal, scavenger activity, and human actions;[c]
- The effectiveness of interventions to reduce transmission and/or geographic spread of the disease; and
- The population-level and economic impacts of CWD and the effectiveness of different interventions to reduce those impacts.

The committee will write a report that addresses these points based on its review of published and in-progress research on CWD.

[a] Free-ranging cervids are wild and not confined by human-made barriers.
[b] Captive cervids include wild animals confined by human-made barriers and farmed cervids confined by human-made barriers and that may be bred.
[c] Human actions include carcass handling, transport and disposal management, live animal transport, and fodder source and transport.

The committee's charge does not include developing recommendations regarding CWD, nor does it include a discussion of the state of knowledge regarding transmission of CWD to humans. During discussions with the committee, the study sponsors indicated that this report will be used to inform discussions of the eventual CWD task force formed under the ACE Act and its decisions regarding an action plan for consistent and coordinated management of CWD. The audience for this report includes state, provincial, tribal, and federal wildlife and captive cervid managers and decision makers; conservation and hunting groups, the captive cervid industry; individuals and groups interested in CWD and cervid management; members of Congress; and the public at large (J. Malmberg, presentation to the committee, October 9, 2023).

COMMITTEE COMPOSITION

Eleven experts were convened specifically to deliberate the task described in Box 1.2. Expertise on the committee included veterinary medicine and cervid health, prion biology and ecology, pathology, wildlife epidemiology, wildlife management, captive cervid management, husbandry, statistical and spatial modeling, transmission modeling, natural resource economics, testing methodologies, genetic resistance, and transmission surveillance. A range of perspectives was sought, including those with public sector experience in the state (or provincial) and federal governments, experience managing other wildlife diseases, academic researchers, and those familiar with different interested and affected groups and perspectives. Committee member biographies are included as Appendix A.

APPROACH TO TASK

The committee regards CWD as fitting the criteria to be considered among a category of diseases described as emerging infectious diseases (EID). EIDs are defined as newly recognized introduced, or evolved diseases, or those that have recently and rapidly changed in incidence or expansion in geographic, host, or vector range (Petersen, E., N. Petrosillo, and M. Koopmans. 2018). The steady and impressive identification of CWD cases across the United States over the last few decades in captive and free-ranging cervids is consistent with other EID. EIDs have been characterized by the interaction of multiple factors and drivers that converge to create a complex and dynamic environment enabling the emergence or expansion of a disease (IOM, 1992; 2003). The dynamics of CWD are impacted by the convergence of factors at the animal, ecosystem, and human interface adding to its complexity and disease management challenges**.** Thinking of CWD as an EID allowed the committee to view CWD through a comprehensive scientific framework that helps to better understand both the disease and its prevention and control.

This report uses the term "CWD" in reference to the infectious North American phenotype of CWD unless noted otherwise. In addition to exploring the scientific literature related to CWD, the committee heard from its sponsors—three agencies with different mission statements—and from a variety of researchers, from cervid farmers, representatives of the North American Deer Farmers Association, representatives from a derivative products industry, from representatives of state-level wildlife management agencies, and from representatives of a small number of tribal nations during its open-session information gathering meetings (see Appendix B for a list of public meeting agendas). The committee gathered public data where available online and reached out to representatives from several state-level agricultural agencies responsible for captive cervid agencies. CWD-related data are collected and stored by different entities (e.g., government agencies at different levels) for different purposes and are not broadly available to aggregate and analyze. The committee received presentations and information from some agencies and cite that information throughout this report. The committee requested various types of data (e.g., CWD surveillance data) from state agricultural agencies and the USDA but the data were either unable to be aggregated, were unavailable, or not provided for other reasons unspecified to the committee.

In deliberating its charge, the committee focused on the scientific knowledge base, but considered how it was complemented by or conflicted with other understandings, and how conflicts in understanding affect the interventions put in place to control CWD. The committee found it helpful to think about CWD knowledge in terms of where information might fall in a knowledge matrix in which knowledge falls into 4 categories: the "known knowns", the "unknown knowns", the "known unknowns" and the "unknown unknowns" (see Box 1.3). Parsing specific aspects of knowledge (or lack thereof) about CWD into one of these quadrants could make it easier to assess the relative availability of information for decision-making, the likely expedience of filling knowledge gaps, and the potentially impactful avenues for research.

BOX 1.3
Organizing Knowledge

A U.S. military strategic framework termed the "known-unknown matrix"[a] provides a way to think about the state of knowledge for CWD. The matrix may help explain the uncertainties and knowledge gaps that characterize this disease. CWD knowledge fits into one of the four quadrants of the matrix. By categorizing and defining relevant knowledge and information about CWD, decision-makers can separate known facts from unsupported information and help determine where scarce resources may best be invested, even when there are substantial uncertainties. Much can be learned but conclusions may be hampered by information in the third quadrant—the unknown knowns. The implicit knowledge in this category could be based on excellent research and surveillance activities, but in many cases the data are inaccessible. The current knowledge base will be expanded when knowledge is shifted from quadrants II, III, and IV to quadrant I.

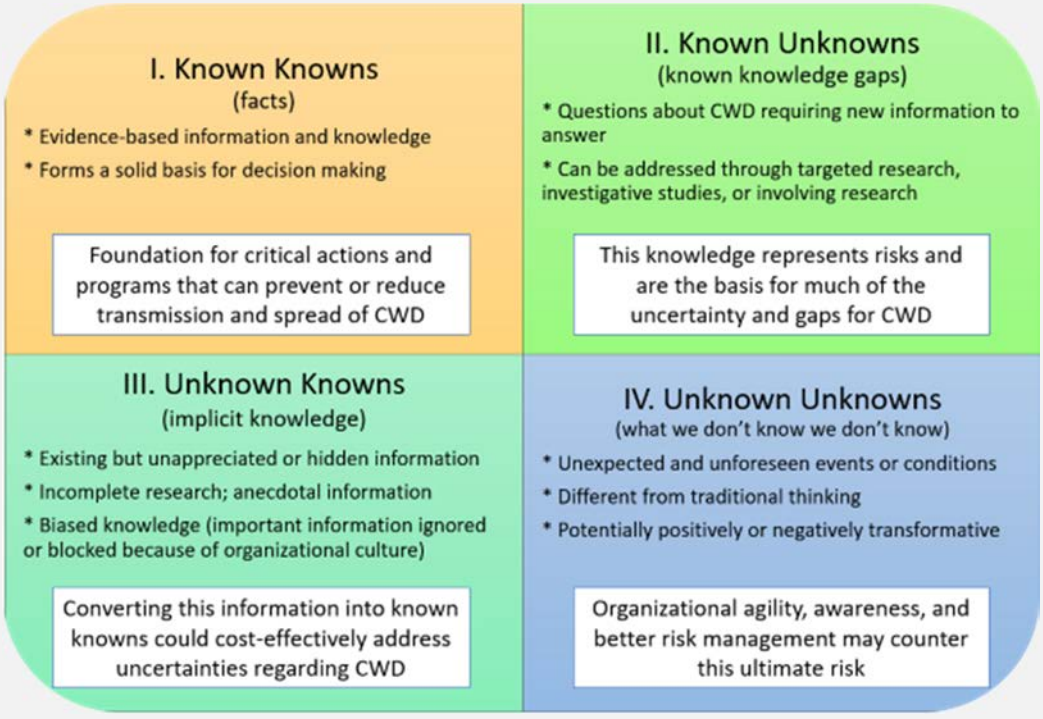


FIGURE 1-3-1 The knowledge matrix in which knowledge fits into one of four categories: known knowns, known unknowns, unknown knowns, and unknown unknowns.
SOURCE: Committee generated.

[a] Department of Defense news briefing by Donald Rumsfeld, February 12, 2002. See https://web.archive.org/web/20160406235718/http://archive.defense.gov/Transcripts/Transcript.aspx?TranscriptID=2636 (accessed April 2, 2024).

THE UTILITY OF KNOWLEDGE

There are numerous groups with interest in CWD, each with its own perspectives and priorities. Table 1.1 provides examples groups and their perspectives. None of these groups can be described as monolithic. The "state of knowledge" regarding CWD is different for each of these groups, and each of the groups will have a different understanding CWD and different approaches and priorities regarding CWD management. An agency employee charged with managing wildlife may view CWD differently than an employee charged with regulating cervid industries. Someone focused on control of an infectious disease may view CWD in still a different way. Each will collect and manage information differently, and each might even have a unique vernacular. As such, deliberating the statement of task (Box 1.2) was challenging for the committee.

Although publicly available data from federal and state agencies related to CWD transmission, spread, and even on the number of cervids in wild and captive populations are limited, there is a good body of published or otherwise accessible knowledge. There is also an apparently large amount of tacit knowledge regarding CWD among scientists and animal health, wildlife management, and cervid industry entities (i.e., knowledge based on unpublished information). There are also non-scientific interest groups with their own understanding and tacit knowledge about CWD. These groups can sometimes be distrustful of scientific information. These different knowledge bases can be contradictory. Table 1.1 provides examples of sectors, constituents, and perspectives that have interest in or knowledge about CWD that might be considered when developing CWD management plans.

TABLE 1.1 Examples of Sectors and Constituents with Interest in CWD and their Perspectives

Sector	**Constituents**	**Perspectives**
Government Agencies Federal Tribal State/province Local	Wildlife management Captive cervid management Infectious disease management	Control of CWD transmission/spread in wildlife settings; Ecological conservation; Control of CWD in captive settings; Revenue generation; Prevention of spread of infectious disease to other species/humans
Native American/First Nations/Indigenous peoples	574 Federally-recognized Native American Tribes in the U.S. (Schwartz, 2024) State-recognized Native American Tribes Native American communities First Nations	Cultural and economic uses of cervids; Food sovereignty, security, and safety; Federally-protected treaty rights Investment by Tribes in natural resource stewardship
Captive cervid industries	Breeding Meat production Commercial products (*e.g.*, urine, antlers, leather) Zoos (for- or non-profit) Product consumers	Revenue generation; Cultural/family importance; Education; Research
Hunters	Active hunters Hunter-related industries • Tourism • Transportation related • Weapons/ammunition • Auxiliary equipment/products • Taxidermy	Food; Subsistence; Supplemental; Cultural heritage/spiritual; Entertainment/hobby; Social/sport; Revenue generation

continued

TABLE 1.1 *continued*

Sector	Constituents	Perspectives
Non-consumptive interest groups	Park visitors Nature enthusiasts Conservationists	Sustainable wildlife populations; Functioning ecosystems; Watchable wildlife
Scientists	Academic Industry Public sector	Academic pursuits; Industry research and development; Wildlife/Ecosystem management support; Agricultural management support

CWD MANAGEMENT

Responsibilities for CWD prevention, surveillance, monitoring, and control within the United States lie primarily with state and tribal authorities, with some exceptions for federal holdings (e.g., national parks and wildlife refuges). Interstate and international movements of cervids are also regulated under federal authority. Activities related to captive cervids are regulated by various combinations of animal health/agriculture authority and wildlife/natural resource management authority depending on jurisdiction. State-tribal-federal cooperation and collaboration to varying degrees also takes place. This inherent complexity in authority and responsibility for CWD and its animal hosts affects all aspects of addressing this disease, including how knowledge about disease management is gathered, documented, and shared. This diversity presents both challenges to and opportunities for making progress toward CWD control at a national scale.

The ACE Act recognizes management of CWD and its wide-reaching impacts are highly complex issues. Management will involve the interplay of, and input from, a variety of interest groups from an array of disciplines, including animal, human, and environmental health professionals, wildlife biologists, laboratory researchers, law enforcement personnel, sociologists, economists, local, state, federal, and tribal leaders, and often members of the public—everyone from nature enthusiasts to sanitation workers. The management of the original severe acute respiratory syndrome (SARS) caused by the first identifiable strain of the SARS-related coronavirus (SARS-CoV-1) in 2003 also required the interplay of an array of groups and experts. The success was at least partially the result of the application of "One Health" concepts,[3] forming the basis of what would become The Manhattan Principles (2004)—the 12 recommendations for holistic prevention of epidemic[4] or epizootic diseases, protecting the integrity of ecosystems and ecosystem diversity, and benefiting people, and domesticated animals. The One Health approach allows the development of solutions based on consideration of disease effects on a range of human, animal, environmental, social, economic, and political interests to develop solutions (see Figure 1.2). With rare exception,[5] CWD solutions have been challenging to develop and implement. Widely accepted consensus on solutions often have been impossible to reach due to numerous interdependent and often contradictory factors. CWD could be considered a "wicked" problem—one that is complex and often intractable with no single definitive solution. Like other wicked problems, improved understanding of CWD and its appropriate management will require genuine collaboration between diverse groups to develop long-term strategies and uncover mutually agreeable solutions, to a degree far beyond what has been demonstrated to date.

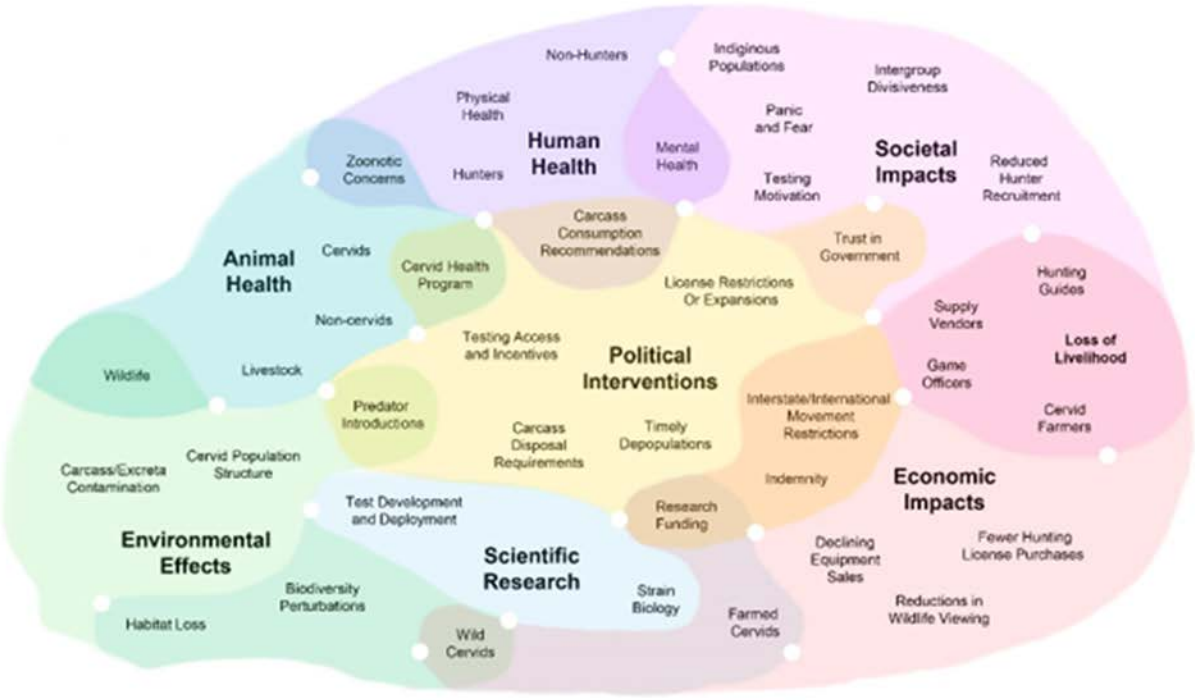


FIGURE 1.2 Because CWD impacts not just the deer, elk, and other cervids that are infected, but also the environment, human populations, and many associated interest groups, its management necessarily involves a consideration of each of these components. The complex interplay between divergent interest groups, social forces, and animal, human, and environmental health make CWD a perfect example of a "wicked problem."
SOURCE: Committee generated.

REPORT ORGANIZATION

This report is organized into eight chapters, with Chapters 2-7 including a header box of bulleted chapter highlights. Chapter 2 provides a brief history of CWD in North America followed by descriptions of CWD, and the state of knowledge regarding CWD strains, infectious doses, incubation period, clinical presentation, disease pathogenesis and infectivity. Chapter 3 provides discussion of transmission mechanisms and routes of exposure, potential host range, and the potential for spillover of the disease into other animal species and humans. Chapter 4 focuses on diagnostic and surveillance methodologies and strategies followed by a discussion of the epidemiology and ecology of CWD in Chapter 5, including a discussion of the impact of CWD on cervid population dynamics. The effectiveness of interventions to control or reduce the spread of CWD in captive and free-ranging cervid populations is addressed in Chapter 6, and Chapter 7 provides a description of the state of knowledge with respect to the human and socioeconomic dimensions of CWD. Chapter 8 synthesizes the committee's overarching conclusions regarding the state of knowledge regarding CWD. The conclusions are intended to inform the design of a strong action-oriented and integrated strategy to reduce the transmission and further geographic spread of CWD. The report is appended with answers to questions commonly asked by the lay public. This is provided as Appendix C.

2
CWD Description, Pathogenesis, and Infectivity

Chapter Highlights

- Chronic wasting disease (CWD) is an emerging infectious prion disease affecting certain species of deer, elk, moose, and other cervids. Infection causes "spongiform" degeneration in the brain and is considered to be fatal to the host.
- CWD prions are misfolded prions similar to those that cause transmissible spongiform encephalopathies (TSEs) in other animal species. The modes of transmission and management of different TSEs differ in some respects.
- The exact origin of CWD remains unclear, but disease transmission models suggest CWD has likely been on the North American landscape for decades prior to first documented occurrences in many locations given incomplete early surveillance efforts**.**
- There are multiple strains of CWD, but understanding of strain emergence, evolution, and prevalence is challenging because current prion strain evaluation techniques do not allow a detailed analysis of the abnormal protein structure. Comparison of data from different studies is often not possible because of the lack of data regarding host cervids and their locations.
- CWD prion infectivity has been detected in various tissue types, with the highest concentrations found in the brain and lymphoid tissues.
- Relatively little is known about the true minimum dose for natural routes of exposure. Estimates derived from experimental studies may suffice as benchmarks for additional in vitro studies to better understand transmissibility. Applicability of future findings would benefit from emphasis on the use of infectious samples from cervids as well as shared reference standards and well-characterized assays.
- Incubation periods vary depending on host genetic background (including, but not limited to the *PRNP* gene), the prion strain, the route of administration and the animal species involved, but challenge experiments focused solely on *PRNP* background indicate the most common genotypes (i.e., 96G/96G in white-tailed deer and 132M/132M in elk) have shorter incubation periods.

CHRONIC WASTING DISEASE

Chronic wasting disease (CWD) is not caused by bacteria, virus, or other more familiar infectious agents, but instead is caused by misfolded versions of prions that are normally found in the bodies of cervids (see Box 1.1). Like other prion disorders, CWD is the result of infection with a misfolded variant of the host's normal cellular prion protein, PrP^{C} (see Box 2.1 for a list of acronyms related to prion proteins). The gene that encodes the normal prion protein in cervids—*PRNP*—shares common structural features with both bovine (i.e., cattle) and ovine (i.e., sheep) prion protein genes (see Box 2.2 for a technical description of the structure of the prion protein and Table 2.1 for a comparison of the "classical" forms of prion diseases of hoofed stock in the United States). Although the *PRNP* gene shows sequence similarities across cervid species, there are several allelic variations (i.e., nucleotide changes in the DNA) that encode prion proteins with different amino acid sequences (Robinson et al., 2012). A subset of these variations is linked to slower CWD progression (Figure 2.1 and Table 2.2) and extended incubation times, although comparatively less is known about disease pathogenesis in cervids with these genotypes compared to those with more common genotypes. Of the genotypes evaluated to date, none confer

complete resistance to prion infection.[1] Because susceptibility to CWD is considered polygenic, other genes likely have a role in both susceptibility and pathogenesis. This concept is discussed in Chapter 6.

Prion diseases are the result of an infection with one or more different prion strains, defined as conformational variants of infectious prions, which may be transmitted between hosts or arise spontaneously in an individual host. Multiple CWD strains have been identified (Otero et al., 2023). Although a spontaneous form of CWD is suspected in some Scandinavian cervids (Pirisinu et al., 2018; Ågren et al., 2021), spontaneously occurring CWD cases have not been described in North America to date. Currently, clinical patterns of disease resulting from the varied CWD strains are difficult to differentiate from one another, and disease pathogenesis largely parallels what is seen with sheep scrapie. Both the extent and levels of prion accumulation in the central nervous system and peripheral tissues has been well studied and may allow discrimination between various CWD strains and spontaneously arising forms of the disease.

CWD occurs naturally in some deer (*Cervidae*) species, in both captive and free-ranging populations. This includes mule deer (*Odocoileus hemionus hemionus*), black-tailed deer (*Odocoileus hemionus columbianus*), white-tailed deer (*Odocoileus virginianus*), North American elk (*Cervus canadensis*), and moose (*Alces alces*). The infectious form of CWD also has been observed in both captive[2] and experimentally inoculated (Mitchell et al., 2012) reindeer (*Rangifer tarandus*; sometimes called caribou in North America), and in captive red deer (*Cervus elaphus*), Sika deer (*Cervus nippon*, Sohn et al., 2020), and sika/red deer hybrids (Prion 2016 Animal Prion Disease Workshop Abstracts, 2016). Two phenotypes of the disease (i.e., the way the disease presents itself in the host) have been described—distinguished by whether abnormal prion protein (PrP^{CWD}) accumulates in the lymphoid tissues of infected cervids (Tranulis et al., 2021; EFSA Panel on Biological Hazards, 2023). The lymphoid-associated CWD form ("phenotype") is most common and, to date, is the only phenotype described in North America (EFSA Panel on Biological Hazards et al., 2023). Ample experimental and epidemiological data support the notion that the phenotype of CWD occurring in North America behaves as an infectious disease in susceptible cervid hosts.

This chapter provides an overview of CWD in North America, beginning with a brief history of its emergence and geographical expansion across the continent. It examines the various prion strains and their role in infection. The chapter also explores key factors such as infectious dose for disease transmission, incubation period, and clinical presentation of infected cervids. In addition, the chapter details the pathogenesis of CWD and prion distribution in various host tissues.

BOX 2.1
Acronyms Related to Prion Proteins

PrP^{C}: normal host prion protein found in all mammals

PrP^{Sc}: abnormally folded, aggregated forms of the prion protein (can be infectious); can also **specifically** refer to the abnormal protein in scrapie-infected sheep

PrP^{CWD}: the misfolded prion protein in CWD-affected cervids

***PRNP*:** refers to the gene that encodes the prion protein

[1] Note: this report uses the term "disease resistance" to imply reduced susceptibility to disease in a manner that is common usage in agricultural settings and related scientific literature.
[2] See https://www.aphis.usda.gov/sites/default/files/usaha-annual-cervid-health-report-2018.pdf (accessed August 20, 2024).

BOX 2.2
Structure of the Prion Protein

The normal prion protein in cervid species is 256 amino acids in length, with sequence and structural similarities to the bovine and human prion proteins (see Figure 2-2-1). The most common prion gene form in white-tailed deer, the 96G haplotype (i.e., a group of closely linked genes on a chromosome that are inherited together), encodes a protein that shares a 94% similarity with that of cattle and 89% similarity with the human prion protein, primarily in the highly structured C-terminal end of the protein. The remaining N-terminal structure is more flexible and carries some importance regarding structural misfolding (Guadagno and Medina, 2023; Safar et al., 1993).

The amino acid sequence of the abnormal prion protein associated with CWD, PrP^{CWD}, is identical to the normal cellular prion protein, except that the protein itself is improperly folded. Helical structures in the C-terminus take a predominantly pleated sheet structure, which has a role in the misfolded proteins highly stable nature (Caughey et al., 1991). Because of the insoluble and aggregating nature of abnormal prions, electron microscopy of the CWD prion structure has been challenging; however, highly sensitive imaging methods (cryogenic electron tomography and transmission electron microscopy) provided images of rodent-adapted scrapie (Kraus et al., 2021) and L-type BSE (Kamali-Jamil et al., 2021) strains, respectively. Both studies confirmed the pleated sheet structure of misfolded prions and could provide insight into CWD prion propagation and strains.

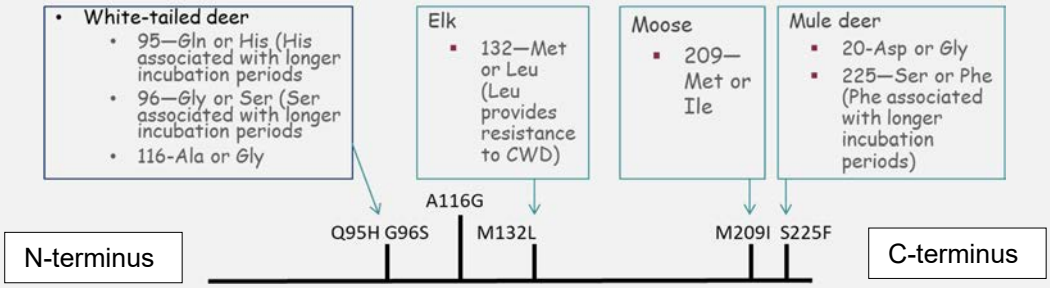


FIGURE 2-2-1 Schematic representation of the host prion protein (PrP^{C}) showing the location of important amino acid polymorphisms. The numbers denote the amino acid modified. Amino acids are denoted by either their 3 letter or single letter code (Gln or Q=glutamine; His or H = histidine, Gly or G = glycine; Ser or S = serine, Ala or A = Alanine; Met or M = Methionine, Leu or L = leucine; Ile or I = isoleucine; Phe or F = phenylalanine; Asp or N = asparagine). The Asp/Gly variant in mule deer is not shown on the protein as this amino acid is located in the signal peptide at the N-terminus of the protein and is not usually present in the mature protein.
SOURCE: Committee generated.

BRIEF HISTORY OF CWD IN NORTH AMERICA

As described in Box 1.1, a clinical syndrome consistent with what has come to be known as CWD was documented in the United States in 1967 in Colorado and recognized as a spongiform encephalopathy in 1978 (Williams and Young, 1980, 1992). The cause of the emergence and initial spread of CWD within North America remains obscure, poorly understood and untraceable to a single point of emergence with any reliability (Williams and Young, 1992; Williams and Miller, 2003; Miller and Wolfe, 2023). Epidemiological models of CWD that incorporate diagnosed cases of the disease in free-ranging and captive cervids indicate that the disease was likely present in some areas of Colorado and Wyoming at least two decades prior to its initial classification as a transmissible spongiform encephalopathy (TSE) (Miller et al., 2000; Williams and Miller, 2002). Similarly, other disease transmission models suggest CWD may have been present in free-ranging white-tailed deer in Wisconsin at least 30 years before its discovery there in 2001 (Wasserberg et al., 2009), somewhat complicating the general supposition that the disease originated from a singular occurrence in western cervids.

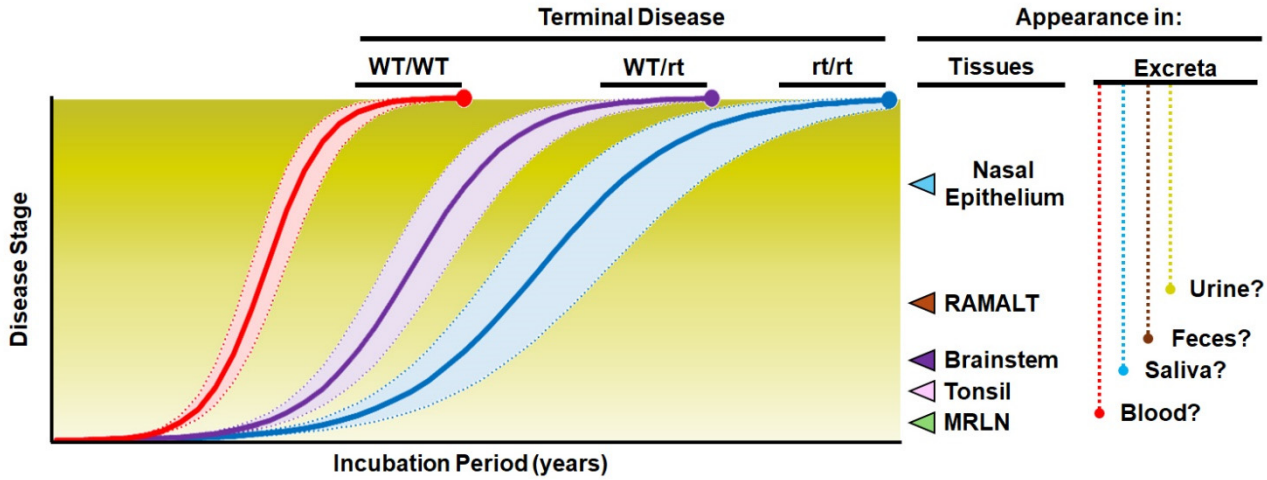


FIGURE 2.1 Model of the pathogenesis of CWD in cervids with variations in the *PRNP* gene. Extended incubation periods in animals with recessive-type (rt) *PRNP* alleles (e.g., 96S in white-tailed deer and 132L in elk; see also Table 2.2) compared to those with wild-type (WT) alleles (e.g., 96G in white-tailed deer and 132M in elk). Variability in animals with common *PRNP* type may be partially explained by genetic differences, outside of the *PRNP* gene, contributing to CWD susceptibility and pathogenesis. Variations in *PRNP* have also been linked to a delay in the appearance of CWD prions in tissues and bodily fluids ("excreta") commonly targeted for diagnostic testing, though the exact timing of accumulation and appearance in excreta from animals carrying recessive *PRNP* genotypes is not well understood. RAMALT: recto-anal mucosa associated lymphoid tissue; MRLN: medial retropharyngeal lymph node.
SOURCE: Figure adapted from Haley and Richt, 2017.

Across its geographical and host range, CWD occurs as several prion strains (discussed in the next section) and the two distinct phenotypes (lymph-associated and non-lymph-associated (reviewed by Miller and Wolf, 2023). This strain and phenotype diversity, along with the known pathogenesis of other TSEs, lend plausibility to three primary hypotheses of CWD's origins:

- An event, or events, of spontaneous prion protein misfolding in individual cervids may have led to the development of an infections prion disease that then spread to other cervid populations and species (Williams and Young, 1980, 1992; Kahn et al., 2004; Tranulis et al., 2021; Williams, 2005, Williams and Miller, 2003).
- CWD may have adapted over time in cervids in one or more locations after direct or environmental exposure to a strain of scrapie, a TSE affecting domestic sheep (Williams and Young, 1992; Williams and Miller, 2003). This theory is often favored given that CWD and scrapie are unique among the TSEs in their ability to transmit horizontally via animal-to-animal contact as well as via environmental contamination (Tranulis et., 2021). Both early (e.g., Colorado/Wyoming) and recent (e.g., Norway) CWD outbreaks have overlapped with domestic sheep travel routes and pasture. Additionally, CWD can be transmitted to domestic sheep via intracerebral inoculation, and white-tailed deer have been successfully inoculated with classical scrapie proximately of U.S. origin via oronasal (mouth and nose) exposure (Greenlee et al., 2023).
- CWD may have developed from an unidentified strain of TSE in an unknown free-ranging or captive mammal species before spreading to other cervid species (Miller and Wolfe, 2023; Williams, 2005).

The ultimate source (or sources) and emergence date(s) of CWD will likely remain unknown given the absence of definitive data.

It would be inaccurate to view the arc of CWD geographical expansion and increase in prevalence as a linear progression of the disease through time and across landscapes. Even prior to its formal documentation, cervids exhibiting clinical signs consistent with possible CWD had been observed in multiple cervid research facilities and zoos in both the United States and Canada (Miller and Wolfe, 2023). As recognition of the disease increased in the late 1990s and early 2000s, so did efforts to implement surveillance programs throughout North America. Early surveillance programs were not well coordinated, often too broad in geographic scope for the limited sample sizes, and inconsistently applied to reliably document the emergence, presence, or spread of CWD in free-ranging or captive herds within a particular area (Miller and Fischer, 2016). CWD surveillance will be discussed further in Chapter 4. Unfortunately, misunderstanding about the limitations of early surveillance data by media outlets and organizations disseminating information about CWD[3] has given rise to an impression among the public and even wildlife management professionals that each discovery of CWD was viewed as evidence that the disease was rapidly spreading (Miller and Fischer, 2016; Ruder, Fischer, and Miller, 2024). However, it is likely that many "new" incidences of CWD were simply the first documented cases discovered because of increased surveillance and do not likely represent the first occurrence of CWD on the landscape. Subsequent sampling from some supposed "new" foci typically revealed many more cases, indicating that the disease had been in the local herd or area for many years or even decades (Miller and Wolfe, 2023; Miller and Fischer, 2016).

CWD's true expansion at local and global scales has been facilitated by both anthropogenic (i.e., originating because of human activities) and natural factors, exacerbated by the long pre-clinical phase of CWD infection (cervids appear healthy throughout most of the disease course). For example, pre-clinical (healthy-looking) CWD-infected captive elk from South Dakota were transported to captive cervid facilities in Saskatchewan and then to South Korea, resulting in the first known cases of CWD in elk in Canada and in South Korea (Kahn et al., 2004; Sohn et al., 2002). Extensive and often undocumented anthropogenic movement of captive cervids before and after the disease's discovery likely has facilitated two-way, horizontal transmission between affected captive and wild populations, as has the escape or release of exposed captive cervids (Miller and Wolfe, 2023; Miller and Fischer, 2016). However, not all distal CWD outbreaks can be attributed to anthropogenic spread of infected cervids or cervid parts. The 2016 discovery of CWD in Norwegian reindeer has no documented or even plausible link to an anthropogenic spillover event from North America, and recent identification of novel strain types in Europe suggest that these strains of CWD may have arisen independently or in parallel to North American variants (Nonno et al., 2020; Tranulis et al., 2021). Though unconfirmed as definitive modes of disease transmission, the movement of CWD-infected cervid parts by hunters, commercial sale of goods containing infected cervid products, and the transportation of hay and animal feed contaminated by infectious CWD prions from soil, feces, and cervid parts may have played some role in the disease's increase in prevalence and geographic footprint (Miller and Fischer, 2016).

Regardless of the initial transmission modality, once established in free-ranging cervids, CWD spreads to new areas and populations adjacent to the initial disease foci and increases in prevalence within infected cervid herds. The natural ability to transmit via shedding of infectious prions into soil and other elements of the environment (Miller et al., 2004) including direct contact between infected and susceptible cervids (Henderson et al., 2015a; Mathiason et al., 2006; Denkers et al., 2024), facilitated spread of CWD within and between cervid herds and species across large areas of North America and, independently, parts of northern Europe. Natural migration, social interactions, mating and other cervid behaviors (e.g., grooming, maternal interactions, congregation around food and water sources), may have played a significant role in the past and current expansion of CWD in free-ranging cervids in affected populations (Miller and Wolfe, 2023; Miller and Fischer, 2016). Box 2.3 provides a brief history of CWD in Canada.

[3] See https://e360.yale.edu/features/chronic-wasting-disease-deer (accessed August 20, 2024).

BOX 2.3
The History of CWD in Canada

In the early and mid-1980s, CWD was translocated inadvertently in farmed elk and white-tailed deer imported from the United States to various farms in southern and central Saskatchewan (Bollinger et al., 2004). The disease then established itself in local wild deer populations adjacent to infected farms. The index case in wild deer in Canada was a mule deer (*Odocoileus hemionus*) harvested in Saskatchewan in fall 2000 (Bollinger et al., 2004). The disease subsequently spread along primary east-west river valleys (Nobert et al., 2016) and spilled out of Saskatchewan into eastern Alberta (detected 2005) and western Manitoba (detected 2021). Based on current levels of surveillance, CWD occurs throughout parkland and prairie habitats of central and eastern Alberta, southern Saskatchewan, and southwestern Manitoba along the border with Saskatchewan.[a] It was recently detected in southeastern British Columbia (2024), adjacent to an affected area in northern Montana.[b] It occurs primarily in mule deer, particularly males, and white-tailed deer, with spillover into sympatric individual moose (*Alces alces*) and elk and with increasing risk of potential spillover into caribou (*Rangifer tarandus*) along the southern fringe of the boreal forest in northern Alberta and Saskatchewan (Alberta Fish and Wildlife and Saskatchewan Environment, unpublished). Initially CWD in farmed elk and deer was associated primarily with movements and interchange of cervids among farms in the prairies (Argue et al., 2007; Baker, 2022). However, as CWD increased in wild deer populations, movement of farmed cervids did not align with disease occurrence on farms and risk investigations suggest wild deer, local forage, or grain supplements are a likely source of transfer into recent cervid farms in both Alberta and Saskatchewan (Canadian Food Inspection Agency [CFIA], unpublished data).

[a] See https://www.usgs.gov/media/images/distribution-chronic-wasting-disease-north-america-0 (accessed August 20, 2024).
[b] See https://www.usgs.gov/media/images/distribution-chronic-wasting-disease-north-america-0 (accessed September 20, 2024).

PRION STRAINS

Like viruses and other pathogens, prions can exist as different strains—traditionally defined as an isolate that produces a unique pattern of neuropathology when passaged under controlled experimental conditions (Fraser and Dickinson, 1967; Fraser and Dickinson, 1968). The concept of strains, as applied to prions, was first suggested in 1961 based on the different clinical manifestations experienced by scrapie-infected sheep (Pattison and Millson, 1961), and later demonstrated through transmission experiments in laboratory rodents (Bruce, 1993). While most pathogen strains are associated with changes in their nucleic acid genomes, prions exist in a range of different conformations (i.e., "shapes" of individual prion strains can be different); it is these variations that drive the differences in observed neuropathology (Bessen and Marsh, 1992; Hill et al., 1997; Safar et al., 2015). Unfortunately, practical laboratory discrimination of prion strains is difficult given that current techniques for strain typing (such as lesion profile analysis, PrP deposition and transmission properties) do not allow a detailed analysis of PrP^{Sc} structure, resulting in an incomplete understanding of both their molecular characteristics, and their importance in disease ecology. The precise mechanisms underlying the generation of prion strains are not yet clear but are affected by both the amino acid sequence of the host *PRNP* and the prion conformers present in the infected individuals (Bartz, 2016). Because of the potential for novel and diverse strains to appear in a single host, they may independently result in novel pathology or alter the susceptible host range of the original prion (reviewed in Bartz, 2016). The isolation of two distinct strains of hamster-adapted transmissible mink encephalopathy in outbred hamsters provides evidence that host background likely does not play a role in the generation of strains (Bessen and Marsh, 1992). Strains have been identified in numerous animal species, including classical and atypical scrapie in sheep and goats (Pattison and Millson, 1961; Benestad et al., 2003), classical and atypical BSE in cattle (Wells et al., 1987; Casalone et al., 2004), sporadic types and variant Creutzfeldt-Jakob disease (Parchi et al., 1996; Parchi et al., 1997; Parchi et al., 2000; Bruce et al., 1997; Hill et al., 1997), and CWD in cervids (Angers et al., 2010; Otero et al., 2023).

Early transmission studies of CWD isolates into transgenic mice (e.g., "bioassay" using mice genetically modified to replace the mouse prion gene with the prion protein from another species) first suggested a potential for strain variation (Browning et. al., 2004; LaFauci et al., 2006). Further bioassays in hosts expressing different PrP^C sequences confirmed the existence of multiple CWD strains (Angers et al., 2010; Perrott et al., 2012; Duque Velásquez et al., 2015; Duque Velásquez et al., 2020; Herbst et al., 2017; Hannaoui, Schatzl, and Gilch, 2017; Moore et al., 2020). CWD strains can have different characteristics in experimental hosts, with host range and *PRNP* sequence variations playing an important role in CWD strain emergence (Duque Velásquez et al., 2015; Duque Velásquez et al., 2020; Hannaoui, Schatzl, and Gilch, 2017; Moore et al., 2020). Complicating the understanding of strain emergence and prevalence is the lack of information in early CWD studies regarding species or genetic makeup of the individuals from which transmitted isolates were prepared. Given the evolutionary potential of CWD strains, specifying the year the sample was collected, as well as additional metadata like the age, sex, species, and location of the animal, is vitally important to enable comparison of data among independent studies.

INFECTIOUS DOSE

Understanding the "minimum infectious dose" of exposure to CWD prions is important for understanding an individual animal's risk of developing the disease. The minimum infectious dose for some bacterial agents (e.g., *Francisella tularensis*) may be as low as a single bacterium (Jones et al., 2005), while some viral agents such as norovirus have minimum infectious doses approaching 20 viral particles (Hall, 2012). For many disease agents, determining a minimum infectious dose is done in either natural (e.g., deer) or experimental (e.g., mice) model systems. Given the difficulties housing and maintaining wild cervids over long periods and given questions regarding the suitability of experimental mouse models for informing the understanding of CWD in natural hosts, determining a minimum dose of CWD prions required for infection of cervids has been challenging. To date, a single study in captive white-tailed deer provides information regarding the minimum infectious dose for CWD via oral inoculation (Denkers et al., 2020; see Box 2.4), although other studies over the past two decades, discussed below, provide additional boundaries on the understanding of infectivity and insights into a correlation between dose and incubation period. A range of factors likely affect minimum infectious dose, including the volume of inoculum and the route of exposure, disease stage of the donor (i.e., the animal from which the infectious prions were collected), amount of infectious prions in the dosing substrate (e.g., brain, blood, or saliva), and other organic or inorganic modifiers (e.g., clay, organic acid), as well as cervid species, age and genetic background of the target host, including both *PRNP* genetic makeup and other potential markers of susceptibility, as discussed in Chapter 6 (Natural and Artificial Selection).

BOX 2.4
Determining the Minimum Infectious Dose of CWD Prions in Deer

To determine preliminarily a minimum infectious dose for CWD, researchers orally inoculated white-tailed deer with increasingly diluted preparations of either brain or saliva from CWD-positive white-tailed deer (Denkers et al., 2020). The level of prions (e.g., the "titer") in each preparation was known—an important aspect of future studies looking at the minimum CWD infectious dose. Although the *PRNP* genotype of each deer was reported, a limitation of the study was that other potential genetic markers of susceptibility were not investigated. Each of four deer given 1 milligram of infectious material developed CWD by 19 months. The response to increasingly smaller doses led researchers to conclude that the minimum infectious dose approximates 300 nanograms (ng) of brain – provided as a single dose or in three separate 100 ng doses. Cumulative inoculation of smaller doses (e.g., 10 inoculations at 30 ng each) to a total of 300 ng did not cause infection, suggesting that the cumulative titer of prions administered may not be as important as the titer of prions in an individual dose. A minimum volume of 15-25 milliliters of saliva, with roughly the same in vitro seeding activity (discussed on Chapter 4) as 300 ng brain, was also found to be infectious.

Box 2.4 describes an experimentally derived minimum dosage of approximately 300 nanograms (ng) of brain equivalents or 15-25 ml of saliva that successfully infected white-tailed deer, resulting in clinical disease 21-38 months following exposure (e.g., the "incubation period" of the disease). A retrospective review of experimental infections over the past several decades indicates that the doses provided were often significantly higher than this estimated minimum infection dose. In white-tailed deer and mule deer, for example, oral doses commonly ranged from 5-10 grams (g) of brain homogenate (Sigurdson et al., 1999; Fox et al., 2006; Johnson et al., 2011b)—equating to perhaps15-30 million times the predicted minimum infectious dose. Similar dosing protocols were used in other cervid species, including moose (Kreeger et al., 2006), reindeer (Mitchell et al., 2012), and sika deer (Sohn et al., 2020). Interestingly, approximately 5g of CWD-positive brain homogenate administered via the oral route was sufficient to cause sub-clinical infection (i.e., PrP^{CWD} were replicated in the brains but there were no clinical signs of disease) in pigs—a species not known to be naturally susceptible to CWD (Moore et al., 2017). Although it is possible that these high doses, compared to more "natural" levels of infectivity, could confound understanding of CWD susceptibility, pathogenesis and shedding, experimental infections of white-tailed deer with high doses of CWD prions had similar incubations periods to naturally infected deer (Johnson et al., 2006a).

Other prion sources (besides brain) and infection routes have been investigated. For example, when white-tailed deer were intravenously injected with 100-250 milliliters (mL) of blood from CWD-positive mule deer, they developed CWD within approximately 2 years (Mathiason et al, 2006). Oral inoculation studies using relatively small volumes (0.1-1.0g) of brain or tonsillar tissue found that incubation periods largely mirrored those of cervids infected through direct intracranial inoculation or larger oral doses (Mathiason et al, 2006, Miller et al., 2012; Wolfe et al., 2012). In contrast, oral inoculation of urine and feces—with volumes ranging from 50-85 mL and 50-112 g, respectively—failed to induce clinical infection after 18-19mos (within the range of a natural infection), although these animals were later found to be sub-clinically infected (Mathiason et al, 2009; Mathiason et al, 2006, Haley et al., 2009a). Environmental exposure, although difficult to quantify, commonly resulted in infection within 1-2 years (Mathiason et al., 2009; Miller et al., 2004; Moore et al., 2016). Relatively little is known about the true minimum dose for natural routes of exposure. However, estimates derived from experimental studies may suffice as benchmarks for additional in vitro studies to better understand transmissibility. Applicability of future findings would benefit from emphasis on natural routes and sources of infection (i.e., oral exposure, environmental exposure using inocula from infected animals/tissues)**,** as well as use of common and well-characterized assays and reference standards.

Across model systems, both incubation period and attack rates (i.e., the proportion of animals infected when exposed to a certain dose) may be influenced by inoculation dose, including cross-species transmission studies (Mammadova, Cassmann, and Greenlee, 2020; Baier et al., 2003; McLean and Bostock, 2000; Collins et al., 2005; Fryer and McLean, 2011). Although this may also be true in cervids (Denkers et al., 2020; Wolfe et al., 2012), there is currently little data to confirm this observation in all cervid species (Hamir et al., 2006a; Moore et al., 2018). Similarly, disease progression, and in some cases prion tissue distribution, in various animal models may be affected by different dosing protocols (Race, Oldstone, and Chesebro, 2000; Herzog et al., 2004).

As differences in tissue distribution can occur between the various cervid species (possibly dictated by strain or genetics), the timing and intensity of shedding in urine and feces may possibly be affected (Denkers et.al, 2024)**.** Without having a more accurate correlation between natural exposure and experimental doses, understanding these features of CWD pathogenesis in both natural and alternate hosts is problematic (Mathiason et al., 2009; Moore et al., 2016).

INCUBATION PERIOD

The incubation period of CWD (i.e., preclinical stage of disease prior to onset of clinical signs) may differ widely across various *PRNP* genotypic backgrounds—and likely other genetic sites—within and between cervid species (see Figure 2.1). Initial experimental studies focused on animals with the most

common *PRNP* genotypes in captive and free-ranging animals, including the highly susceptible 96G homozygous genotype (i.e., having two identical versions of the same gene—one from each parent) of white-tailed deer, mule deer, and reindeer, and the 132M/132M genotype of elk (see Table 2.2). Time to onset of clinical disease following oral inoculation in these studies was typically 1.5-2.5 years in white-tailed deer, mule deer, and reindeer (Fox et al., 2006; Johnson et al., 2011b; Miller et al., 2012; Mitchell et al., 2012), and up to two years in elk (Moore et al., 2018), similar to incubation periods reported for scrapie in sheep (Spickler, 2016). At present, there is no indication that the oral exposure dosage affects incubation period. In white-tailed deer, incubation periods following more unnatural routes of exposure, including intracranial, intravenous, and intraperitoneal inoculation, largely paralleled those of oral exposure (Mathiason et al., 2006; Mathiason et al., 2009).

Recently, attention was given to those *PRNP* genotypes that, based on past studies, suggest differential susceptibility, including the 96S genotype of white-tailed deer, the 225F genotype of mule deer, the 132L genotype of elk, as well as several alternate genotypes in reindeer (O'Rourke et al., 2004; Jewell et al., 2005; Johnson et al., 2003; Johnson et al., 2006a; O'Rourke et al., 2007; Mitchell et al., 2012). In those studies, the onset of clinical signs was delayed compared to more common *PNRP* genotypes; in some cases, incubation periods approached 5 years or more (Johnson et al., 2011a; Miller et al., 2012). Further studies investigating both susceptibility and incubation periods in white-tailed deer with more rare, heterogenous *PRNP* backgrounds are currently underway (e.g., USDA Agricultural Research Service, 2021).[4]

In contrast to experimental inoculations where the timing of exposure is known, the incubation periods arising from natural CWD infections cannot be measured with precision, especially in the wild. However, incubation periods in experimental oral inoculation studies largely matched those estimated from field data in free-ranging animals (e.g., Miller et al., 2008; Edmunds et al., 2016; DeVivo et al., 2017). The youngest detected wild deer and elk with clinical CWD were 16 and 21 months, respectively (Spraker et al., 1997; Williams and Miller, 2002), well within the range of data from experimental inoculations. Available evidence thus suggests the incubation after natural exposure is probably not substantively different from what has been produced experimentally, and consequently what has been learned from experimental studies can inform understanding of natural disease processes. In addition to the demonstrated variation explained by *PRNP* genotypic background discussed above, it seems plausible that other host, agent, or environmental factors also could affect incubation after natural exposure.

With the discovery of a novel form of CWD in Scandinavia characterized by the absence of lymphoid PrP^{CWD} and all cases involving relatively old animals (Pirisinu et al., 2018; Vikøren et al., 2019; Tranulis et al., 2021), it is evident that the causative strain may also be a factor in CWD incubation period.

CLINICAL PRESENTATION

The clinical progression and presentation of CWD and can differ within and between species (Fox et al., 2006; Johnson et al., 2011a; Williams and Young, 1980). Clinical features—most apparent in experimentally infected animals—include changes in behavior and progressive deterioration of body condition (i.e., weight loss) (Williams, 2005). Signs of disease progress over weeks to months following disease onset (Williams, 2005). Cervids showing signs may display changes in how they interact with herd mates or their handlers, and may display fixed stares, repetitive movements, periods of somnolence, and hyperexcitability when approached. Their postures may appear altered, with lowered head and ears, and arching of the back, and there may be ataxia (Fox et al., 2006; Johnson et al., 2011a; Williams and Young, 2000). Changes in gastrointestinal microbial composition have been suggested in infected white-tailed deer, although further comparisons of affected and unaffected animals are warranted to better understand whether such changes may serve as disease markers (Didier et al., 2024; Minich et al., 2021). Advanced clinical disease may involve bruxism (teeth grinding), polydipsia (excess drinking), polyuria

[4] See https://www.ars.usda.gov/research/project/?accnNo=440677 (November 6, 2024).

(excess urination), difficulty swallowing, regurgitation of rumen contents, and excess salivation with drooling. As is reported for similar protein misfolding disorders, progression of neurologic disease eventually leads to recumbency, aspiration pneumonia, dehydration, or hypothermia during the winter season, which can ultimately result in the death of the infected animal (Williams and Miller, 2000). Compared to deer, captive elk with CWD can display nervousness and hyperesthesia (increased sensitivity) and are more likely to display gait changes and less commonly polydipsia (Williams, 2005). Although these clinical signs are less apparent in free-ranging animals, the subtle progression of early neurologic disease makes infected deer and elk more likely to be hit by cars (Krumm, Conner, and Miller, 2005), killed by predators (Krumm et al., 2010; Miller et al., 2008; DeVivo et al., 2017), or harvested by hunters (Conner, McCarty, and Miller, 2000; Edmunds et al., 2016; DeVivo et al., 2017) than their uninfected counterparts.

PATHOGENESIS OF DISEASE

Figure 2.1 is a timeline model of the pathogenesis of CWD in cervids with different *PRNP* genotypes over time, with disease stage (discussed in detail later in this section and in Chapter 4) representing the accumulation of prions in different bodily tissues (Spraker et al., 2023, 2004; Keane et al 2008a; Fox et al., 2006; Haley and Richt, 2017). The distinguishing pathological characteristics of CWD in deer are similar to those of scrapie-infected sheep and other prion diseases acquired through ingestion of infectious material. In orally infected deer, PrP^{CWD} crosses the intestinal epithelial barrier and can be detected in lymphoid tissues associated with the alimentary tract (e.g., within one-month post-exposure can be detected in lymphoid tissues associated with the alimentary tract such as the gut-associated lymphoid tissue; GALT), as well as tonsils and retropharyngeal lymph nodes (RPLN) within one month of exposure (Fox et al., 2006; Hoover et al., 2017a; Sigurdson et al., 2001; Sigurdson et al., 1999). Within these sites, PrP^{CWD} is found to associate with specific intra- and extracellular target proteins (Sigurdson et al., 2002). This cellular targeting during early phases of the disease suggests that prions cross the mucosa of the gastrointestinal tract and are transported to Peyer´s patches (clusters of lymphoid follicles in the intestines) and regional lymph nodes (Sigurdson et al., 2002; Sigurdson et al., 1999). Prions can then enter nerve endings of the enteric nervous system (ENS) and leak into the lymphatic and blood systems spreading to other organs (Heggebø et al., 2003; van Keulen, Bossers, and Zijderveld, 2008).

Prion infection of the ENS spreads through sympathetic and parasympathetic nerves to the central nervous system (CNS) (Van Keulen, Vromans, and Zijderveld, 2002). An early site of PrP^{CWD} accumulation within deer CNS is the dorsal motor nucleus of the vagus nerve (DMNV), suggesting this nerve as the major route for PrP^{CWD} from the gastrointestinal tract to the brain (Sigurdson et al., 2001). A second route of prion migration into the brain is through retrograde transport of prions up the spinal cord (Kaatz et al., 2012; McBride et al., 2001). Although this route is important during invasion of the brain by bovine spongiform encephalopathy (BSE) prions in cattle, it does not appear to be a critical route of CWD prions in deer. This suggests that accumulation of PrP^{CWD} in the thoracic spinal cord of orally infected cervids results from the spread of CWD prions produced within the CNS (Sigurdson et al., 2001).

Multiple organs may be infected via transport by lymph and blood (see Table 2.3 for examples of studies). Several blood cell types from CWD-infected deer have demonstrated significant prion infectivity, suggesting that the blood-borne spread of infection is likely an important component of disease progression (Mathiason et al., 2010).

Although the early lymphoid replication phase is particularly important for CWD prions (Hoover et al., 2017a; Sigurdson et al., 2001), exceptions were noted in North American elk as well as Scandinavian moose and red deer, where PrP^{CWD} may accumulate in the brainstem with minimal or no accumulation of PrP^{CWD} in lymphoid tissues (Pirisinu et al., 2018; Race et al., 2007; Spraker et al., 2004; Vikøren et al., 2019). This could be explained by a predominantly neural route of entering the brain, sporadic misfolding of PrP^{C} (i.e., similar to atypical scrapie or BSE), and differences in the route of exposure or in strain. Dfferences in route of exposure may also affect prion accumulation in different tissues (Haley et al., 2011). Once in the brain, PrP^{CWD} accumulates in the CNS producing lesions

associated with prion diseases, including intraneuronal vacuolation (large vesicles in the cytoplasm of neurons), spongiosis (spongy appearance of the brain), gliosis (inflammation a nonspecific production or enlargement of glial cells following CNS injuries) and formation of amyloid deposits (Williams and Young, 1993).

In addition to lymphoid tissues, CWD prions can be found in a range of tissues outside the brain, including nasal mucosa, salivary glands, urinary bladder, pancreas, kidney, intestine, and reproductive tract of female and male deer (Fox et al., 2006; Haley et al., 2011; Kramm et al., 2017; Nalls et al., 2017; Otero et al., 2019; Sigurdson et al., 2001) and elk (Spraker et al., 2004; 2010; 2023). CWD prions have also been identified in gestational tissues of pregnant deer and elk (Nalls et al., 2017; Selariu et al., 2015). The accumulation of PrP^{CWD} in some of these tissues is associated with shedding of prions through secretions and excretions (Haley et., 2011). As described for sheep scrapie (Andréoletti et al., 2000) and as noted above, host *PRNP* genotype can influence CWD pathogenesis in deer, notably affecting PrP^{CWD} deposition in peripheral tissues (Fox et al., 2006; Johnson et al., 2011a; Hoover et al., 2017a; Maddox et al., 2020; Otero et al., 2019).

Because the natural progression of clinical disease may be variable in susceptible species, disease staging provides a subjective but reproducible approach for quantifying the progressive accumulation of PrP^{CWD} in those central and peripheral tissues described above (Spraker et al., 2004, Keane et al., 2008a). Often, the RPLN and obex region of the brainstem—the primary tissues collected for on postmortem testing—are the focus of disease staging efforts, though other tissues, including spinal cord, tonsil and other lymphoid tissues, and non-neural tissues may be considered as well (Spraker et al., 2023). Little is known about the correlation between disease stage and the onset of shedding. To date, a uniform approach to disease staging has not been developed, although there are significant areas of overlap between those scoring systems described. A more comprehensive understanding of the alignment of disease stage and the shedding, as reported for other infectious neurologic diseases like rabies (Vaughn, Gerhardt, and Newell, 1965), will be important in aiding disease management strategies.

PRION DISTRIBUTION IN HOST TISSUES

In some prion diseases like BSE, the presence of misfolded prions may be limited to nervous tissue and a narrow selection of "specified risk materials" such as the eyes, tonsils, and distal intestinal tract (USDA, 2019). CWD prions, however, are distributed across many tissues beyond the central nervous system and lymph nodes, including a wide range of peripheral tissues—an important consideration when developing, for example, interstate carcass movement regulations (Gassett, 2019). Table 2.3 is a list of tissues in which CWD has been detected in different cervid species. CWD prion detection in tissues is based primarily on immunohistochemical analysis or *in vitro* amplification methods (see Chapter 4), which provide insufficient information regarding levels infectious prion present (e.g., titers). However, few tissues have been bioassayed in transgenic mice. Little is known about the distribution of CWD prions in North American moose; primarily due to the relatively few cases identified to date. CWD prions also are shed in urine, feces, and saliva.

TABLE 2.1 Comparison of the "Classical" Forms of Prion Diseases of Hoofed Stock in the United States

		Prion Disease ("classical" form)		
Aspect	**Epidemiological feature**	**Chronic Wasting Disease**	**Scrapie**	**Bovine Spongiform Encephalopathy**
Agent	More than one prion strain identified	yes	yes	no
Host	Main host species	deer, elk (Family Cervidae)	sheep, goats (Family Bovidae)	cattle (Family Bovidae)
	Genetic influence on host susceptibility	moderate	strong	none
	Naturally transmitted to unrelated species	not reported	not reported	yes
	Can be experimentally transmitted to unrelated species via oral route	yes	yes	yes
	Can be transmitted to humans	not reported	not reported	yes
Transmission and geographic spread	Transmitted via ingesting infectious tissues	yes (experimental)	yes	yes
	Infectivity in tissues beyond central nervous system	yes	yes	no
	Transmitted via infectious excretions and secretions.	yes	yes	no
	Main driver(s) of transmission within herd	exposure to infectious animals and environments	exposure to infectious animals and environments	feed contaminated with infectious cattle byproducts
	Main (known) source(s) of introduction into a herd	infectious animals	infectious animals	contaminated feed
	Other potential source(s) of infection	suspected	none reported	none reported
	Persistence of infectivity in the environment	yes (>2 years)	yes (>15 years)	not reported
Control	Examples of successful control (scale)	limited (local)	yes (national)	yes (national)
	Current (ca. 2020s) distribution in the US	widespread	limited/absent	absent
	Historical distribution in the US (ca. 1950-2000)	extent uncertain	widespread	rare
	Outbreaks in free-ranging hosts	yes	not reported	not reported
	Effective vaccines or medical treatments available	no	no	no
	Genetic selection as a control tool	some potential	yes	not needed
	Likely extent of control feasible in the US (given current knowledge and tools)	manageable in captive herds, less so in the wild and as cases and spatial extent increase	eradicable	establishment prevented

SOURCE: Committee generated.

TABLE 2.2 *PRNP* Variations found in North American cervids

Species	*PRNP* Variant	Frequency in farmed +/- free ranging cervids	Influence On: Infection Odds Ratio	Influence On: Disease Progression	Notes	Example References
White-tailed deer	96G	~70%	Reference baseline	Reference standard	Wildtype (WT) genotype; using extended nomenclature: 95Q/96G/116A/226Q	
	96S	~25%, With higher frequency in e.g., Texas	<0.50 (heterozygous) <0.10 (homozygous)	Slows	Using extended nomenclature: 95Q/96S/116A/226Q	Johnson et al., 2003; Johnson et al., 2006a; O'Rourke et al., 2004; Kelly et al., 2008; Wilson et al., 2009; Keane et al., 2008b; Brandt et al., 2015; Miller et al., 2012; Haley et al., 2019; Seabury et al., 2020
	95H	<2%	<0.30 (heterozygous) Homozygous unknown	Slows	Using extended nomenclature: 95H/96G/116A/226Q	Johnson et al., 2011a; Seabury et al., 2020 Haley et al., 2019
	116G	<3%, primarily in Canadian herds	<0.50 (heterozygous) <0.01 (homozygous)	Slows	Using extended nomenclature: 95Q/96G/116G/226Q	Haley et al., 2019; Hannaoui et al., 2021
	226K	<4%, primarily in U.S. herds	<0.60 (heterozygous) Homozygous unknown	Similar to 96G, or only modestly slows	Using extended nomenclature: 95Q/96G/116A/226K	Seabury et al., 2020; Haley et al., 2019
Mule deer	225S	~85%	Reference baseline	Reference baseline	Amino acid sequence identical to the 96G variant in white-tailed deer	
	225F	~5%	<0.1	Slows	PrP^{CWD} present, but may not stain with monoclonal antibody F99/97.6.1; homozygotes rare in natural populations even where CWD is prevalent	Jewell et al., 2005; Wolfe, K.A. Fox, and Miller, 2014; LaCava et al. 2021
	20G	~9%	Similar to 225S	Similar to 225S	Polymorphism in signal sequence, not present in mature protein	Wilson et al., 2009; Jewell et al. 2005
Elk	132M	~75%	Reference baseline	Reference baseline	Amino acid sequence identical to the 96G variant in white-tailed deer except for a Q226E substitution	

	132L	~25%	<0.70 (heterozygous) <0.40 (homozygous)	Slows	132L homozygous elk-different PrP^{CWD} profile on WB	O'Rourke et al., 1999; Perucchini et al., 2008; Hamir et al., 2006a; O'Rourke et al., 2007; Haley et al., 2018; Haley et al., 2019
Moose	209I	~55%	Unknown	Unknown		
	209M	~45%	Unknown	Unknown	Amino acid sequence identical to the 96G variant in white-tailed deer	(Huson and Happ, 2006)
Reindeer/ Caribou	GSV	~40%	Reference baseline	Reference baseline	Using extended nomenclature: 129G/138S/169V/225S; amino acid sequence identical to the 96G variant in white-tailed deer	Mitchell et al., 2012; Arifin et al., 2020;Güere et al., 2019; Moore et al., 2016
	138N	~50%	Variable dependent on zygosity	Variable dependent on zygosity	Using extended nomenclature: 129G (or S)/138N/169V (or M)/225S	Mitchell et al., 2012; Arifin et al., 2020; Moore et al., 2016
	SSM	<2%	Considered less susceptible	Unknown	Using extended nomenclature: 129S/138S/169M/225S	Mitchell et al., 2012; Arifin et al., 2020; Güere et al., 2019
	225Y	Unknown; reported only in European reindeer	0.2 as a heterozygote	Unknown	Using extended nomenclature: 129G/138S/169V/225Y	Güere et al., 2019

NOTE: G = glycine; S = serine; H = histidine; K = lysine, F = phenylalanine; M = methionine; L = leucine; I = isoleucine; N = asparagine; V = valine; Y = tyrosine.
SOURCE: Committee generated.

TABLE 2.3 Detection of CWD Prions in Tissues and Excreta of CWD-infected Cervids

	Tissue	White-Tailed Deer	Mule Deer	Elk	References
Diagnostic Tissues	Brain	Y[a]	Y	Y	Williams, 2005
	Retropharyngeal Lymph Node	Y	Y	Y	Williams, 2005
	Palatine Tonsil	Y	Y	Y	Wild et al., 2002; Hille et al., 2019
	Recto-anal mucosa-associated lymphoid tissue (RAMALT)	Y	Y	Y	Keane et al., 2009; Geremia et al., 2015; Monello et al., 2014; Haley et al., 2016b
	Third Eyelid	Y		Y	Cooper et al., 2019
Non-Diagnostic Peripheral Tissues	Adrenal Cortex			Y	Spraker et al., 2023
	Adrenal Medulla	Y	Y	Y	Sigurdson et al., 2001; Spraker et al., 2023; Otero et al., 2019
	Antler Velvet			Y	Angers et al., 2009
	Embryonic/Fetal Tissues	Y		Y	Nalls et al., 2021; Bravo-Risi et al., 2021; Selariu et al., 2015
	Fat		Y		Race et al., 2009a
	GALT	Y			Hoover et al., 2017a
	Heart	Y		Y	Henderson et al., 2015b; Jewell et al., 2006; Spraker et al., 2023
	Ileum	Y		Y	Hamir et al., 2008; Spraker et al 2023
	Interdigital Glands	Y	Y		Ness et al., 2022
	Kidney	Y[b]		ND[c]	Haley et al., 2011; Otero et al., 2019; Spraker et al., 2023
	Liver	N		ND	Otero et al., 2019; Spraker et al., 2023
	Lung	Y		ND	Otero et al., 2019; Spraker et al., 2023
	Mammary Gland			Y	Spraker et al., 2023
	Myenteric Plexus		Y	Y	Sigurdson et al., 2001; Spraker et al., 2023
	Ovaries			Y	Spraker et al., 2023
	Pancreatic Islets	Y[b]	Y	Y	Spraker et al., 2023; Otero et al., 2019; Sigurdson et al., 2001
	Peyer's Patches	Y	Y	Y	Sigurdson et al., 1999; Hamir et al., 2006a; Otero et al., 2019
	Pituitary Gland	Y	Y		Otero et al., 2019
	Placenta	Y		Y	Bravo-Risi, F et al., 2021; Selariu et al., 2015
	Proximal Colon	Y		Y	Spraker et al., 2023; Haley et al., 2011
	Retina	Y		Y	Keane et al., 2008a; Spraker et al., 2010
	Salivary Glands	Y[b]		Y	Haley et al., 2011; Otero et al., 2019; Spraker et al., 2023
	Skeletal Muscle		Y	Y	Angers et al., 2006; Spraker et al., 2023
	Spinal Cord		Y	Y	Spraker et al., 2023; Spraker et al., 2002a
	Spleen	Y	Y	Y	Henderson et al., 2015a; Race et al., 2007

	Tissue	White-Tailed Deer	Mule Deer	Elk	References
Excreta and bodily fluids	Blood	Y			Kramm et al., 2019; Mathiason et al., 2006
	Cerebrospinal Fluid	Y		Y	Nichols et al., 2012; Haley et al., 2013
	Feces	Y[b]	Y	Y	Haley et al., 2011;Tamgüney et al., 2009; Tewari et al., 2022; Plummer et al., 2017; Henderson et al., 2017
	Saliva	Y			Henderson et al., 2015a; Haley et al., 2009b
	Semen	Y			Kramm et al., 2019
	Urine	Y[b]			Henderson et al., 2015a; Haley et al., 2009b; Plummer et al., 2017

[a] Y designates the presence of CWD prions.
[b] indicates variable depending on *PRNP* genotype of the species (Spraker et al., 2023; Race et al., 2007; Race et al., 2009a; Sigurdson et al., 2001; Balachandran et al., 2010; Otero et al., 2019; Plummer et al., 2017)
[c] ND designates samples were tested but CWD prions were not detected.

3
Mechanisms of Transmission and Potential Host Range

Chapter Highlights

- CWD is transmitted by multiple mechanisms, but the oral-nasal route is thought to be the most common.
- CWD prions can persist bound to soils, plants, and other surfaces for at least two years and possibly up to decades in the environment where they can be a risk for other cervids.
- CWD transmission is complex and multifactorial. Key factors include but may not be limited to variations in host susceptibility and genetic makeup, differences in CWD prion strains, long persistence outside the host, variability in prion shedding, concentrations of the prions, and the potential roles of vectors and materials that can carry the CWD prions. There are considerable knowledge gaps about the roles and interrelationships of all these factors.
- The role of scavengers and predators in the transmission, spread, and suppression of CWD has been postulated but remains undetermined.
- While there is no evidence currently that CWD can naturally infect animals other than cervids, the possibility of a "spillover" event is a concern. Variation in prion strains associated with CWD could contribute to changes in its natural host range.
- There are no reported human prion diseases linked to CWD. Given the geographic expansion of CWD and the rising number of cases in cervids, the risk for human exposure to diverse CWD strains continues to increase. The probability that CWD could become zoonotic is unknown, but the species barrier is likely high.

Of the naturally occurring prion diseases identified to date, chronic wasting disease (CWD) is singular in its transmissibility and spread among captive and wild cervid populations. The route(s) of transmission have not been determined definitively but are accepted generally to involve either oral or oral-nasal routes of exposure. As with all prion diseases, the incubation period has two separate stages, an extensive (months to years) pre-clinical phase in which the animal appears healthy, followed by a short (weeks to months) clinical phase prior to death. Shedding of infectious prions begins during the preclinical stage and correlates with the accumulation of misfolded prions in various tissues. Vertical transmission of prions from female to fetus before (*in utero*) or shortly after birth has been described, but their contribution to CWD epidemiology is uncertain. Once shed in bodily wastes or through the decomposition of infected carcasses, prions effectively contaminate the environment, including soil, water, plants and persist for extended periods of time.

This chapter focuses on the mechanisms of CWD transmission. The persistence and movement of infectious prions across different elements of the environment and how susceptible cervids may become exposed are described. Finally, the potential of these infectious particles to induce prion diseases in non-cervid species, including humans, is discussed.

ROUTES OF EXPOSURE

The wide geographic dissemination of CWD can be explained, in part, by the efficient transmission of infectious prions between infectious and susceptible cervids. It is generally accepted that the primary route of exposure in natural settings is oral-nasal based on effective transmission reported through both abrasions in the mouths of captive research cervids (Denkers, Telling, and Hoover, 2011),

aerosolization of CWD prions (Denkers et al., 2013), and oral inoculation with infectious materials in the laboratory setting (e.g., Denkers et al. 2020).

The transmission events leading to CWD have been studied in experimental conditions. White-tailed deer, one of the best studied animal species of CWD prion transmission, are susceptible to the prion infectious agent by multiple routes of exposure, including intracerebral, intraperitoneal, intravenous, oral, and oronasal (Johnson et al., 2011a; Hamir et al., 2011; Denkers et al., 2020; Kincheloe et al., 2021). Although some of these routes (e.g., intravenous, intracerebral) are not practical in terms of natural transmission, they are useful to define the transmission potential of CWD prions, the temporal dissemination of infectious prions in different tissues and excreta, and to determine the minimum quantities of prions needed to induce disease. Experimental infections have demonstrated that as little as 300 ng (nanograms) of infectious prions may result in clinical infection of white-tailed deer via the oral route (Denkers et al., 2020) (see Box 2.4). The oral-nasal route, as stated earlier, is the most likely route for CWD transmission in cervids as CWD prions are available to naive cervids directly via animal-to-animal contact, or indirectly through contaminated environmental fomites (Moreno and Telling, 2018; Zabel and Ortega, 2017).

Another potential route of exposure involves mother-to-offspring transmission (Nalls et al., 2013; Selariu et al., 2015). CWD prions have been detected in fetuses and gestational tissues of naturally and experimentally infected white-tailed deer (Bravo-Risi et al., 2021; Nalls et al., 2021). Fawns born from experimentally infected Reeves' muntjac deer (*Muntiacus reevesi*) dams presented increased risks of prion infection (Nalls et al., 2013). Mechanisms associated with CWD vertical transmission are yet to be defined.

PRIONS IN THE ENVIRONMENT

CWD prions are introduced into the environment to different extents and through different animal products (e.g., urine, feces or tissues released from decomposing carcasses or placenta) containing variable quantities of prion infectivity. Contaminated environments can remain a source of infection for decades or more (Georgsson, Sigurdarson, and Brown, 2006) and can contribute to the dissemination of prions. Infected carcasses may be consumed by mammalian and avian predators or scavengers (e.g., coyotes, vultures, feral pigs), as well as parasitic and non-parasitic invertebrates (e.g., insects, annelids) that may spread prions beyond where the host died (Soto et al., 2024; Pritzkow et al., 2021; Baune et al., 2021; Inzalaco et al., 2024; Nichols et al., 2015). CWD prions are found in soils (Kuznetsova et al., 2024) predator and scavenger fecal material, in internal and external parasites including nasal bots[1] and ticks (Haley et al., 2021b; Inzalaco et al., 2023), as well as in plant material and water (Carlson et al., 2023; Pritzkow et al., 2015; Nichols et al., 2009; Plummer et al., 2018) although infectivity levels are unknown. Plummer and others (2018) detected the presence of CWD in samples collected at or near salt licks. Analysis of soils in and around deer carcass burial pits also resulted in the detection of infectious prions (Soto et al., 2023a; Schwabenlander et al., 2024). Infectious prions were detected in feeders in CWD infected captive facilities (Soto et al., 2023a).

Environmental samples can be at least partially decontaminated in laboratory settings (Kuznetsova et al., 2018), but there are no practical approaches to eliminate prions efficiently and effectively in a contaminated natural environment. Environmental decontamination procedures being studied are discussed in Chapter 6.

Soils

Cervids consume significant amounts of soil both directly and inadvertently. In the lab, prions bind to soil and soil minerals, but binding capacity differs with the composition of the soil or mineral type (Johnson et al., 2006b). Laboratory experimentation has shown that prions bind more avidly to soils and

[1] Nasal bots are the larva of bot flies that are found on the skin of the nose and mouth of white-tailed deer (e.g., https://myfwc.com/research/wildlife/health/white-tail-deer/nasal-bots; accessed April 30, 2024).

clay minerals over time, making recovery increasingly more difficult, although infectivity remains unaffected (Kuznetsova et al. 2020). Prions bind readily to the clay mineral montmorillonite (Mte), and far less to sandy soils such as quartz sand. Areas rich in clay soils display higher CWD incidence compared to areas of other soil types (Walter et al., 2011). Further, when prions are bound to Mte and then assessed for infectivity using bioassay (e.g., exposing lab cervids to a sample suspected to contain infectious prions), the incubation periods are shorter than when using prions not bound to Mte, suggesting that, in some cases, binding to soil or soil minerals enhances infectivity (Johnson et al., 2007).

Migration of prions through soil columns packed with different soil types is also soil-type dependent. When soil columns contain Mte or Mte-rich soils, prions do not migrate and remain near the top of the column. In quartz or sandy soils, prions move through the column, ultimately being detected in materials leached from the column (Kuznetsova et al., 2023). These results suggest that the availability of prions for subsequent transmission via the environment is dependent on soil type. The binding and lack of migration of CWD through clay soils underpins the recommendation that clay liners be used for carcass disposal pits (Jacobson et al., 2010). Differential binding of prions to different soil types—and the variable effect of soil components on prion detection methods—complicates the detection and quantification of CWD prions in the environment (see Chapter 4 for a discussion on detection methods).

Plants

Plants are the main component of cervid diets. Infectious CWD prions can mechanically bind to plants (Pritzkow et al., 2015), although it is important to note that current evidence related to prion-plant interactions comes primarily from research laboratories conducting controlled and proof-of-concept experiments (Pritzkow et al., 2015; Carlson et al., 2023). Grass plants exposed to infectious prions from different sources (including from brain extracts and excreta from experimentally infected cervids) have been found via prion replication analyses PMCA (Protein Misfolding Cyclic Amplification) and RT-QuIC (Real-Time Quaking-Induced Conversion) to retain prions bound to their surfaces even after being extensively rinsed (Pritzkow et al., 2015; Carlson et al., 2023). Plants also can take up infectious prions from experimentally contaminated soil. Infectious prions were detected in the aerial portions of plants (i.e., stems and leaves) by PMCA (Pritzkow et al., 2015) and by fluorescently labelled PrP^{Sc} (Carlson et al., 2023). Experimental exposure of plants to CWD-contaminated soil suggests that edible plants can uptake prions from the soil and transport them to their aerial parts. For example, carrots grown in CWD-contaminated soils contained prions in both the roots and leaves, as detected by PMCA and confirmed by animal bioassay (Soto et al., 2023b). When root and leaf homogenates were individually inoculated intracerebrally into transgenic mice expressing deer prion protein, both roots and leaves resulted in clinical CWD infections (Soto et al., 2023b).[2] However there is no data demonstrating the presence of transmission-relevant concentrations of CWD prions in natural settings nor is there evidence of natural infection transmitted to a cervid via plant tissues.

Determining whether plants in naturally infected environments can serve as reservoirs of infection is a critical question in CWD research. Concerns over the role of foraged crops in the geographic spread of CWD has led some governments (i.e., Norway) to ban the import of forage materials from any jurisdiction where CWD is present.[3]

Parasites

The role of parasites in the transmission of CWD is largely unexplored. Whether parasites can act as vectors of CWD transmission is relevant, considering the wide variety of parasites (e.g., ticks, keds [a type of biting fly], mites, nasal bots, and several species of worms in lungs, stomach, muscles, liver, and

[2] Transgenic studies are those in which genetic material from one species is artificially introduced to that of another species.

[3] See https://lovdata.no/dokument/LTI/forskrift/2018-10-22-1599 (accessed August 21, 2024).

arteries) that can occur on or in cervids. Parasites potentially could serve as vectors of CWD as they are transmitted between individuals, and CWD prions can be present in infectious amounts in cervid tissues during the long pre-clinical phase.

Ticks and nasal bots have been investigated for their potential roles in CWD transmission. Results related to transmission through ticks have been contradictory. Shikiya and others (2020) reported that ticks (*Dermacentor andersoni*) experimentally exposed to prion-infected hamsters could not infect new hamster hosts. These ticks also did not contain any prions, as measured by PMCA. It is important to note, however, that midgut contents present in tick homogenates (most likely blood) may affect detection using prion amplification methods. In contrast, prion seeding activity was demonstrated in winter ticks (*Dermacentor albipictus*) harvested from naturally infected North American elk by RT-QuIC assays (Haley, et al., 2021a) (see Chapter 4 for a description of diagnostic tests). More recently, CWD prions were detected by PMCA and RT-QuIC in lab-fed black-legged ticks (*Ixodes scapularis*) and identified in black-legged ticks collected from naturally infected white-tailed deer (Inzalaco, et al., 2023). The specific infectivity titers in these parasites, *ergo* their relevance for disease transmission, is unknown. The potential for ticks to transmit CWD needs to be carefully evaluated considering the low infectivity titers (i.e., the amount of infectivity in a sample) in blood of the tick's host, the tick species' natural life cycle in vertebrate hosts, the amount of infectivity in the tick, and the potential routes of infection (e.g., through transmission when the tick is consuming a blood meal versus inadvertent consumption of the tick during vertebrate grooming).

Some fly species, including bot flies (*Cephenemyia phobifer*), deposit their eggs in the nostrils of cervids where larvae mature within the nasal cavity and pharyngeal pouches (Soto et al., 2024). These anatomical structures are relevant for CWD as cervids can be readily infected via nasal exposure to CWD prions and prions are shed in nasal secretions. Moreover, cervids can host hundreds of nasal bots at a time (Texas Parks and Wildlife field personnel, personal communication, January 2021). CWD prions can be detected, via PMCA, in nasal bots collected from naturally infected, pre-clinical white-tailed deer (Soto et al., 2024). As the last stages of nasal bot larvae maturation occur in soil, there is also the potential for further contamination of the environment. As parasites do not express the cellular prion protein (PrP^{C}) required for prion replication, they likely bind prions on their surface (Soto et al., 2024). The potential for parasites to serve as disease vectors depends on both their potential to bind prions and their interactions with the contaminated host or other potentially infected components (e.g., blood or other tissues). Animal bioassay studies have suggested that ingestion of a single bot would be sufficient to infect another deer (Soto et al., 2024), however, overall, there are more questions than answers regarding the role of fly larvae in potentially transmitting CWD.

HOST RANGE AND SPILLOVER TO OTHER SPECIES

As the geographic range and disease prevalence of CWD continues to increase, the frequency of exposure of other species to CWD also increases (Otero et al., 2021). The potential for spillover into a new species is dependent on the species barrier effect (the likelihood of infection of a different species) (Hill and Collinge, 2004). Multiple factors contribute to the species barrier and thus impact the likelihood of CWD spillover. These include: (1) among other genetic factors, the amino acid sequences of the prion protein in both source and recipient cervids; (2) titer of the infectious agent; (3) the CWD strain; (4) the route of infection; and (5) possibly, the immune status of the newly infected cervids.

Although other host factors may be involved, the prion protein is critical for prion infection as deletion of the gene encoding the prion protein abolishes the ability of an animal to be infected (Sailer et al., 1994; Richt et al., 2007). Transmission of prion diseases is more likely to occur when there is sequence homology or similarity between the amino acid sequences of the host PrP^{C} and the pathogenic PrP^{Sc} (Prusiner et al., 1990; Hill et al., 1997). For example, although mice present a strong species barrier (i.e., are not readily infected) to several naturally existing prion strains, this barrier can often be overcome by removing the endogenous *PRNP* gene and replacing it with the equivalent gene of the PrP^{Sc}-donor animal species (Browning et al., 2004; Herbst et al., 2022; Angers et al., 2010). Genetic modifications,

particularly in rodent models of prion disease, have allowed identification of the specific amino acids contributing to the species barrier; however, susceptibility/resistance to infection by a specific prion strain can only be determined empirically (Cullingham et al., 2020).

Multiple strains of CWD prions have been identified (see Chapter 2). Different conformations of the misfolded prion protein results in CWD strains with different properties. Although most of the characterization of CWD strains is laboratory-based (i.e., analysis is based on transmission and biochemical properties), strains have been identified in naturally-infected cervids. CWD strains can preferentially infect cervids with different PrP^C sequences, for example, transgenic mice expressing 96S-PrP can be infected with some but not all CWD strains (Duque Velasquez et al., 2015; Hannaoui et al., 2021). Wisc-1, one of the best studied prion strains, can cause clinical disease in mice but not hamsters, while another CWD strain (H95+) can successfully infect hamsters but not transgenic cervidized mice (Herbst et al., 2017). These suggest that different strains may have different host ranges (i.e., the breadth of species able to be infected), impacting potential for transmission to non-cervid species, including humans.

The immune status of the newly infected cervids may impact intra- and inter-species transmission of prions. Several avenues of research suggest that the innate immune system (i.e., the immunity that an organism is born with) provides an early barrier to establishment of a CWD-prion infection. These early defense mechanisms reduce the amount of infectivity in tissues important in the early stages of infection (see pathogenesis section). Cells of the immune system also express considerable levels of PrP^C and act, in non-cervid species, as early sites of prion replication as well as contribute to prion dissemination to different tissues (Blättler et al., 1997; Montrasio et al., 2000; Heikenwalder et al., 2005). Different levels of inflammation in various tissues have been shown to alter the distribution of prions in a positive manner (Heikenwalder et al., 2005; Ligios et al., 2005). Considering this, modifying the immune responses potentially can tip the balance into further preventing infection or allowing infection to occur (Carroll and Chesebro, 2019; Makarava et al., 2024). Although most of the experimental data, to date, is from laboratory animals, similar responses are anticipated to occur in cervid species. As many other pathogens will also activate the innate immune system, the status of the immune system in an individual animal may be predictive of whether an infection can be established.

Experimental Inter-Species Transmission

The potential transmission of CWD prions to non-cervid animal species is of great concern as inter-species transmission of prions is known to favor the emergence of novel prion strains with new infectious potentials and host ranges (Bartz et al., 1998; Morales et al., 2007; Morales, 2017; Herbst et al., 2017). There is no evidence of CWD spillover resulting in clinical disease in non-cervid species in nature. In the laboratory, however, transmission experiments often utilizing direct brain inoculation suggest that multiple different species could be susceptible (i.e., be capable of replicating the infectious prions) to CWD (see Table 3.1). In experimental studies, CWD prions have been transmitted to raccoons (Moore et al., 2019), ferrets (Bartz et al., 1998; Sigurdson et al., 2008), cattle (Hamir et al., 2001), sheep (Hamir et al., 2006b), pigs (Moore et al., 2017), squirrel monkeys (*Saimiri sciureus*) (Race et al., 2009b), and multiple North American rodents that share the environment with cervids including meadow voles (Heisey et al., 2010; Carlson et al., 2015), red-backed voles (Carlson et al., 2015; Heisey et al., 2010), white-footed mice (*Peromyscus leucopus*) and deer mice (*Peromyscus maniculatus*) (Heisey et al., 2010). It is possible that these species could serve as reservoirs for CWD, although no evidence of natural occurrences has been reported. Transgenic mouse studies have also demonstrated that beaver may be susceptible to CWD (Herbst et al., 2022).

Several different carnivore species have been infected with CWD in experimental paradigms (Mathiason et al., 2013). Ferrets (*Mustela furo*) are a valuable model for many prion diseases, including CWD. Their susceptibility to CWD could provide information about the crossover potential of CWD to other species (Sigurdson et al., 2008). Mink (*Mustela vison*), on the other hand, are susceptible to CWD only by the intracranial route (Harrington et al., 2008). Oral and intracranial challenge of domestic cats

(*Felis catus*) have resulted in no clinical disease and low attack rates on first passage, but, on second passage, 100 percent of the cats presented with clinical disease following intracranial challenge and 50 percent disease via the oral route (Mathiason et al., 2013). In contrast, extensive, long-term natural exposure of mountain lions (*Puma concolor*) to CWD-infected cervid carcasses failed to result in infection (Wolfe et al., 2022) despite this species' susceptibility to bovine spongiform encephalopathy (Kirkwood and Cunningham, 1994). Transmission to raccoons was CWD strain-dependent, with both elk and white-tailed deer CWD, following intracerebral infection, showing low attack rates while mule deer CWD did not transmit (Cassmann et al., 2022, Moore et al., 2019, Hamir et al., 2007).

It is important to recognize the limitations associated with experimentally testing the species barrier to CWD infection. Testing species barrier in experimental studies generally relies on intracranial inoculation of the infectious agent. This route circumvents many of the clearance mechanisms thought to reduce prion titers initially (Chang et al., 2024) and results are interpreted as proof-of-concept to assess susceptibilities. Thus, successful transmission via the intracerebral route of exposure does not necessarily mean that infections will occur via other routes (Harrington et al., 2008; Mathiason et al., 2013; Moore et al. 2017; Williams et al., 2018). Unfortunately, some of the most widely used mouse models of prion disease are not susceptible to peripheral routes of exposure (Bian et al., 2019). Oral infection can also be several orders of magnitude less efficient than intracranial infections requiring exposure to higher amounts of infectivity (e.g., Moore et al., 2017).

TABLE 3.1 Experimental transmission of CWD to different animal species. SOURCE: Otero et al., 2021.

Species	Route of CWD Transmission[a]	References
Cervids		
Muntjac deer *(Muntiacus reevesi)*	IC, PO, SC	Nalls et al., 2013
Fallow deer (*Dama dama*)	IC	Hamir et al., 2011
North American caribou (*Rangifer tarandus caribou)*	PO, environmental	Mitchell et al., 2012; Moore et al., 2016
Livestock		
Cattle *(Bos Taurus)*	IC	Hamir et al., 2011; Greenlee et al., 2012
Sheep	IC	Hamir et al., 2006b; Mitchell et al., 2015
Pigs	IC, PO	Moore et al., 2017
Non-human primates		
Squirrel monkeys (*Saimiri sciureus*)	IC, PO	Race et al., 2009b
Rodents		
Present in areas where CWD occurs: Meadow voles (*Microtus pennsylvanicus)* Red-backed voles (*Myodes gapperi*) White-footed mice (*Peromyscus leucopus*) Deer mice (*Peromyscus maniculatus*) House mice	IC	Heisey et al., 2010;
Not present in CWD areas: Syrian golden hamster (*Mesocricetus auratus)* European bank vole (*Myodes glareolus)*	IC	Raymond et al., 2007; Di Bari et al., 2013 Herbst et al., 2017
Carnivores		
Domestic Ferrets *(Mustela furo)*	IC, PO, IP	Bartz et al., 1998; Sigurdson et al., 2008; Perrot et al., 2012
Mink (*Mustela vison*)	IC	Harrington et al., 2008
Domestic Cats	IC, PO	Mathiason et al., 2013
Raccoons (*Procyon lotor*)	IC	Moore et al., 2019

NOTE: susceptibility to intracerebral (IC) exposure may not reflect susceptibility to exposure via natural routes.
[a] IC: intracerebral; PO: oral, SC: subcutaneous, IP: intraperitoneal.

Experimental Infection of Livestock

As sheep and cattle are both susceptible to non-CWD prion diseases (scrapie in sheep and BSE in cattle), there is concern that CWD could spillover to agriculturally important species. Although cattle and captive cervids can overlap geographically, there is no evidence that cattle can be infected with common strains of CWD prions via natural exposure routes (Williams et al., 2018; Gould et al., 2003). Intracranial inoculations of sheep with mule deer CWD prions did result in clinical disease, albeit with long incubation periods and low penetrance (Hamir et al., 2006b). Successful infection of sheep with CWD prions was dependent on the host *PRNP* genotype and the CWD strain (Hamir et al., 2006b). Infection of transgenic mice expressing multiple copies of a specific sheep *PRNP* allele did not result in clinical disease or accumulation of PrP^{Sc} in the brain; the CWD prions were, however, successfully replicated in the spleen. This suggests a subclinical infection of sheep. If these subclinically infected sheep can transmit the prion disease to another sheep (i.e., via shed secretions), it is possible the prions could adapt to the new host—generating a sheep-specific CWD prion with greater ability to infect sheep (Cassmann et al., 2021).

Domestic pigs can also be infected, albeit poorly, with white-tailed deer CWD prions. The data, to date, suggest that the species barrier between cervid CWD prions and pigs is high. Even low levels of CWD transmission in pigs, however, is concerning due to the possibility that feral pigs, which share ranges with cervids, could become a reservoir of CWD prions. CWD prion detection in wild pigs collected from areas where CWD occurs was proportional to the prevalence of prion infected deer in each area of collection (Soto et al., 2023c). Although there was no evidence of clinical CWD infections of wild pigs in that study, subclinical infections were reported in transgenic mice expressing deer prion protein when injected with tissue homogenates from these pigs. These data suggest that CWD prions were present in pig tissues but at very low levels. Mice expressing the pig prion protein (surrogates for pig susceptibility to CWD prions) did not present with prion disease either as clinical or subclinical prion infection (Soto et al., 2023c).

To date, there is no evidence of CWD spillover to the wide variety of species that share the landscape with free-ranging cervids. Comparison of the *PRNP* sequences of various nondomestic bovid species (bighorn sheep [*Ovis canadensis*] and mountain goats (*Oreamnos americanus*]) suggested that these species are potentially susceptible to CWD (Cullingham et al., 2020); however, natural cases have not occurred among bighorns despite opportunity for environmental exposure (Fox et al., 2021).

CWD ZOONOTIC POTENTIAL

The committee's charge (see Box 1.2) does not include discussion of the state of knowledge regarding transmission of CWD to humans. Nonetheless, summarizing a few key points on this topic in the context of host range and potential for spillover to other species seemed appropriate in this report.

There have been a limited number of epidemiological studies on the topic of spillover of CWD from cervids into humans, and these have found no causal links between CWD exposure and increased frequencies of human prion disease (Mawhinney et al., 2006; Olszowy et al., 2014; Abrams et al., 2011; Belay et al., 2001). A review of those studies is included as part of a larger effort by Waddell and others (2017). However, the increasing prevalence of CWD and broader geographic expansion of the disease in cervid herds can increase opportunities for human exposure, as there are more infected cervids that may be handled or consumed. Given that, it is important to recognize that populations with high frequency of cervid consumption (e.g., subsistence hunters) and that have unique traditional practices (e.g., brain-tanning of hides) may be at disproportionate risk of exposure to CWD prions in areas where CWD occurs (Tranulis and Tryland, 2023; Maraud and Roturier, 2021; Parlee et al., 2021). Monitoring and investigations of potential cases for evidence of CWD spillover in the United States are ongoing.

The risk of spillover to humans has also been examined indirectly in experiments using a variety of laboratory models including two non-human primate species, "transgenic" cervids expressing human prion protein, and in vitro amplification assays. Squirrel monkeys, considered universally susceptible to

prion diseases, were susceptible to infection with CWD (Marsh et al., 2005; Table 3.1). In contrast, a study in rhesus macaques—a species considered more closely related genetically to humans than spider monkeys (Goodman et al., 1998)—suggested they were refractory to CWD infection (Race et al, 2009b; Table 3.1).

Most studies using transgenic mice expressing the human prion protein and developed to enhance their susceptibility potential have failed to identify any evidence of CWD transmission, thereby suggesting that the species barrier preventing CWD infection in humans is relatively high (Race et al., 2022; Wilson et al., 2012; Sandberg et al., 2010; Kong et al., 2005; Race, Williams, and Chesebro, 2019). One published study in transgenic mice that overexpress the human prion protein found no evidence of disease but did report PrP^{CWD} amplification in spleen and other peripheral tissues suggestive of some potential for subclinical infections (Hannaoui et al., 2022). A study using transgenic fruit fly models expressing either human or non-human primate prion proteins reported susceptibility to CWD (Thackery et al., 2024). In considering the practical relevance of various transgenic model results, it is worth noting that these studies relied on genetically altered model systems and inoculation routes that bear little resemblance to the circumstances that might surround natural exposure of humans to CWD.

In vitro assays (e.g., cell-free conversion, RT-QuIC, or PMCA; see Chapter 4), developed to assess the ability CWD prions to replicate using normal human prions as a conversion substrate, also suggest that the species barrier is high between CWD and humans (Raymond et al., 2000; Davenport et al., 2015). While an earlier study reported inefficient conversion of normal purified human prion protein by CWD prions (Raymond et al., 2000), a later study found that PrP^{CWD} could convert recombinant human PrP (Davenport et al., 2015). The failure of CWD prions to infect human cerebral organoids, developed using human stem cells further suggests a high species barrier to infection (Groveman et al., 2024). Taken together the collective results of research to date using a variety of molecular and animal models suggest the species barrier between humans and CWD prions is likely high, although perhaps not absolute.

The potential for animal prion agents to infect humans has been recognized (e.g., EFSA 2011; 2015; 2017) since clear evidence emerged that consumption of classical BSE-infected materials resulted in variant Creutzfeldt-Jakob Disease (CJD) (Will et al., 1996; Bruce et al., 1997; Hill et al., 1997). Determining the actual zoonotic risk of CWD in the United States is epidemiologically complex due to the number of CWD and other animal prion strains present currently, the long incubation of prion diseases in humans (perhaps decades) (Bartz et al., 2000; Hill et al., 2003; Huillard d'Aignaux et al., 2002), and the potential for subclinical (silent) infection (Gill et al., 2013; 2020). The identification of spillover to humans would require a statistical increase in human prion disease in a given geographic location, large-scale screening of exposed populations for potential subclinical infections, or the presentation of a prion disease with different clinical symptoms/characteristics or biochemical signature than currently known forms of CJD (e.g., Will and others [1996] provides a historical perspective).

4
Diagnostics and Surveillance

Chapter Highlights

- Several USDA-approved diagnostic tests are used to screen for CWD and are primarily implemented postmortem. These tests yield consistent and good results, are the only tests considered official, and can only be performed by USDA-approved university, state, and federal diagnostic laboratories.
- A group of non-USDA-approved laboratory-based amplification diagnostic tests that may be used for identifying CWD prions in live cervids has different advantages than USDA-approved immunoreactive testing approaches. They are more sensitive, faster, and can handle greater testing volumes. Further validation of these tests is needed before they can be considered for USDA approval.
- Rapid, relatively inexpensive, field-deployable, antemortem tests for early CWD prion detection (especially in the preclinical phase of CWD infections) and for routine environmental testing and surveillance of captive cervids could be useful for informing decisions related to CWD.
- CWD surveillance for both captive and free-ranging herds is resource-intensive, costly, and unevenly implemented in many regions and situations. These factors have impacted the timely understanding of CWD distribution and emergence in the United States in free-ranging herds, however, much has and can be learned about CWD through effective surveillance if surveillance objectives are well defined and if the limitations of the surveillance strategies are understood.
- The development and implementation of effective, sustainable, coordinated surveillance strategies for both captive and free-ranging cervids is key to better understanding the epidemiology and geospatial characteristics of CWD and seems essential to developing improved prevention and control programs at a national scale.

The sensitive and specific detection of chronic wasting disease (CWD) and CWD prions in clinical and environmental specimens is an important component in CWD management across North America and beyond. Extensive research demonstrates that the most accurate marker of any prion infection is the disease-associated prion protein (PrP^{Sc}) (Prusiner, 1982; Guiroy et al., 1991). Accumulation of this misfolded protein is most readily observed in the central nervous system—especially in advanced stages of disease—although it may be found at lower levels in a range of tissues, bodily fluids, and excreta during the course of infection (Henderson et al., 2020; Tewari et al., 2022; Henderson et al., 2015; Hoover et al., 2017a). Primary diagnostic approaches commonly used in state and federal diagnostic laboratories include immunohistochemistry (IHC; Guiroy et al., 1991) and enzyme-linked immunosorbent assays (ELISA; Hibler et al., 2003). These are mainly used with tissues collected after death, primarily from the obex[1] region of the brainstem and the retropharyngeal lymph nodes (RPLN). Because CWD in both captive and free-ranging cervids is considered a notifiable disease by the U.S. Department of Agriculture (USDA), these assays require regulatory approval for their use in diagnostic settings. In contrast, experimental as-yet unapproved diagnostic approaches have been under continuous development since the early 2000s and have been demonstrated to be sensitive for tissues and bodily fluids collected both antemortem (before death) and postmortem (after death). They can be used for non-cervid biologic samples like insects and plants, and environmental samples ranging from soils to surface swabs. Some of these techniques, including protein misfolding cyclic amplification (PMCA; Kurt et al., 2007; Saá, Castilla, and Soto, 2005) and real-time quaking-induced conversion assay (RT-QuIC; Atarashi et al., 2007), have potential for supplementing conventional approved diagnostic approaches.

[1] The obex is a region of the brainstem that narrows and joins the spinal column.

Lack of regulatory approval is partially the result of a lack of coordinated inter-laboratory cross-validation studies. Regardless of the test, diagnostic testing in CWD surveillance requires careful consideration to ensure both cost-efficiency and confidence in detection.

This chapter provides a description of the diagnostic tests currently available for the detection of CWD and their application in surveillance. Discussion is focused on test characteristics (e.g., sensitivity and specificity), advantages and disadvantages, the suitability of each test for different types of samples (biological versus environmental), and how different samples and tests can be used in surveillance. The state of knowledge related to testing of live cervids and new directions in environmental detection and surveillance also are addressed.

SENSITIVITY AND SPECIFICITY

There are distinct ways in which the results of different diagnostic tests may be quantified, although similar terminology applied to the different tests can create confusion. For example, test "sensitivity" may refer either to the lowest quantity of a target that can be confidently detected by an assay (i.e., analytical sensitivity) or to the probability that an assay will accurately characterize an infected animal as positive (i.e., diagnostic or epidemiologic sensitivity) (Saah and Hoover, 1997). The former is critical in fully understanding the lower detection limits of a test, and it is generally assessed before diagnostic or epidemiologic sensitivity is considered. The latter is an important component of test accuracy, which considers an animal's true disease status. Diagnostic specificity is the second important element of test accuracy and refers to the probability of a test to accurately classify an uninfected or healthy animal as negative. High diagnostic specificity means that an animal infected with another pathogen (e.g., bovine tuberculosis), will not test positive on a test designed to detect CWD.

The goal of any diagnostic test is to achieve the highest sensitivity and specificity possible, although no diagnostic test is perfect and there will always be compromises to optimize results in one way or another. A lower sensitivity means that more animals that have the disease of interest will be misclassified as disease-free. A lower specificity means there will be more false positives (i.e., more uninfected animals will incorrectly test positive), which could have regulatory ramifications depending on jurisdictional policies. In many cases, optimizing for one diagnostic criteria (e.g., sensitivity) comes at the cost of reductions in the other (specificity). Thus, it is not uncommon in disease diagnostic testing to utilize a combination of tests that vary in their sensitivity and specificity. For example, a highly sensitive test may be used to screen animals initially, but where false positives are a concern, a second, highly specific test may be used for confirmation. Beyond understanding the sensitivity and specificity of a test, it is also useful for practitioners to understand the positive and negative predictive values of a test: if an animal tests positive, what is the probability that the animal is truly infected or diseased? Understanding this provides information on how well a test will perform when disease prevalence varies across populations. However, such diagnostic criteria (positive and negative predictive value) are less frequently estimated. Appendix D summarizes information available on the diagnostic sensitivity and specificity of different tests for CWD when applied to different biological samples. Box 4.1 provides additional information related to challenges in the estimation of diagnostic sensitivity and specificity.

CURRENT DIAGNOSTIC TESTS FOR CWD

Diagnosis of CWD in cervids has been based on the detection of the misfolded, disease-associated form of the prion protein, PrP^{Sc}. Tests can be categorized by how the abnormal prion proteins are detected (Haley and Richt, 2017): antigen-antibody ("immunoreactive") interactions, laboratory-based ("*in vitro*") amplification assays, or some combination of the two. Table 4.1 lists these tests and describes their testing

BOX 4.1
The "Gold Standard"

In the assessment of diagnostic test performance, the sensitivity and specificity of the test of interest is often measured in comparison to a "gold standard". The "gold standard" refers to the true infection or disease status of the animal. The only reliable way to achieve this standard is to assess diagnostic test performance using animals that have been experimentally infected and their uninfected controls. This is often infeasible, however, and so a more common approach is comparison to another diagnostic test. While the test of comparison is often one that confers high confidence in its outcome (having high diagnostic sensitivity and specificity), it is never perfect, particularly when applied to populations with a heterogenous mix of animals at various stages of infection and disease progression. Thus, assessing the diagnostic performance of a new test against another test becomes a comparison to our best measure of true disease status and not the "gold standard" (Walsh, 2018; Rutjes et al., 2007; Hui et al., 1998). This is an important distinction to recognize when evaluating the diagnostic performance of new tests for CWD. When new testing methodologies are designed to outperform more traditional methodologies (e.g., Miller and Williams 2002), what should the "gold standard" of comparison be? The scientific community currently faces this challenge in the evaluation of new diagnostic tests for CWD. Overcoming the challenge may necessitate higher benchmarks for the "gold standard" (i.e., bioassay) or adoption of methods of test assessment that do not rely on a gold standard of comparison (Picasso-Risso et al., 2022; Wyckoff et al., 2015). The advantages and limitations to these approaches need to be considered, but the CWD scientific community now needs consensus on this issue.

costs, laboratories,[2] and turnaround times. Historically, the diagnosis of prion disease has relied on the detection of PrP^{Sc} in the brain or lymphoid tissues of animals using western blot, IHC, and ELISA (Bolton, McKinley, and Prusiner, 1982; Haley et al., 2017). Amplification assays such as PMCA and RT-QuIC demonstrate greater sensitivity detecting low concentrations of prions in tissues (Table 4.2; McNulty et al., 2019) and allow earlier detection of CWD prions before or after death, including during earlier stages of infection using alternative tissues and bodily fluids. Such tests are useful for rapid screening but disease diagnosis must still be through IHC or ELISA. Further, enhancements to PMCA (e.g., addition of plastic beads or co-factors; Gonzalez-Montalban et al., 2011; Haley et al., 2013) and RT-QuIC (e.g., NAPTA precipitation, addition of iron oxide beads or silica nanoparticles, or sample pre-treatments; Christenson et al., 2023; Denkers et al., 2016; Henderson et al., 2015) improve sensitivity. Newer diagnostic tests using alternate amplification technologies are also emerging (Christenson et al., 2022). Although not used for routine diagnosis and surveillance (they are not feasible for large number of samples considering the costs and time involved), bioassays (e.g., using experimental inoculation in genetically modified mice or other species to detect infectious prions in samples) have also been utilized to confirm prion presence, particularly when infectivity of a sample is in question. Bioassays may be the only definitive method of prion detection, and until amplification-based test results are further validated as accepted reference standards, their use are limited to confirming disease status of conventional test-negative, amplification test-positive samples. None of the above-mentioned tests are designed to evaluate prion infectivity. Finally, several publications have assessed the utility of cell culture systems for prion detection and quantification (e.g., Bian et al., 2010; Thapa et al., 2022). Although this approach may help supplant the need for mouse bioassay, its utility in diagnostics is limited due to a limited number of cervid species and prion strains to which it can be applied.

[2] Because humans are not known to serve as hosts to CWD prions, the CDC currently recommends the that work with CWD prions be conducted in biosafety level 2 (BSL-2) facilities (CDC and NIH, 2020). There are four biosafety levels and BSL2 measures are put in place to protect laboratory workers from "moderate hazards to personnel and the environment" (see https://crsreports.congress.gov/product/pdf/R/R47695; accessed October 8, 2024).

TABLE 4.1 Comparison of Diagnostic Testing Options Available or in Development for CWD

Test	Use	Sample type (PM, AM, EN)[a]	Sample condition	Cost per sample[b]	Turnaround time[c]	Laboratories
Immunoreactive tests						
Immuno-histochemistry (IHC)	Official testing	Generally PM Some AM	Formalin-fixed	$21-42	1-2 weeks	National Animal Health Laboratory Network (NAHLN)
Enzyme-linked immunosorbent assay (ELISA)	Official testing	Generally PM	Fresh	$22-25	8-24 hours	NAHLN
Western blot	Official testing	PM	Fresh	NA*	1-2 weeks	National Veterinary Service Laboratory (NVSL) only[d]
In vitro amplification assays						
Protein misfolding cyclic amplification (PMCA)	Research	PM, AM, EN	Fresh or frozen	~$5 (materials only)	3-21 days	Research
Real-time quaking-induced conversion (RT-QuIC)	Research	PM, AM, EN	Fresh or frozen	~$7 (materials only)	24-72 hours	Research
Minnesota-quaking-induced conversion (MN-QuIC)	Research	PM, AM	Fresh or frozen	~$12 (materials only)	24 hours	Research

NOTE: Experimental assays have not seen general field deployment; true costs have not yet been fully realized.
[a] PM: Postmortem tissues; AM: Antemortem tissues; EN: Environmental samples.
[b] Published prices for IHC and ELISA obtained from two NAHLN laboratory websites (accessed January 30, 2024). Does not include prices applied to hunter-submitted samples. All other estimates are non-commercial pricing and do not include labor costs.
[c] Turnaround times for IHC and ELISA were obtained from two NAHLN laboratory websites (accessed January 30, 2024). These figures include time required for sample preparation, testing, and reporting as a service; other test turnaround times only estimate sample preparation, testing, or pathologist interpretation. ELISA turnaround time may vary based on sample submission volume and availability of commercial reagents.
[d] Western blot is offered by the National Veterinary Services Laboratory (not other NAHLN laboratories) at no cost as an official confirmatory test following a positive IHC test.

TABLE 4.2 Comparison of Prion Detection in a Dilutional Series of CWD+ Cervid Brain Pool

	Cervid brain pool homogenate dilution								
Assay	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Western Blot									
BioRad ELISA									
Mouse Bioassay	ND					*LD_{50}		ND	ND
sPMCA/WB								ND	ND
RT-QuIC	NT	NA							
sPMCA/RT-QuIC	NT								

NOTES: Shading represents relative prion positivity (lighter shading represents lower relative prion positivity and darker shading represents higher relative prion positivity). 10^{-x} notates the dilution of brain pool, with larger negative numbers representing higher dilutions (i.e., less brain) used in each assay.
BioRad ELISA: the USDA official enzyme-linked immunosorbent assay. sPMCA: protein misfolding cyclic amplification. WB: western blot. RT-QuIC: real time quaking induced conversion. ND: dilutions at which bioassays were not done. NT: dilutions not tested by RT-QuIC or PMCA. LD50: the lowest dilution at which 50% of the experimental mice were killed.
Committee note: MN-QuIC had not been developed when this table was published, but information about its sensitivity and specificity can be found in Appendix D of this report.
SOURCE: McNulty et al., 2019.

Immunoreactive Assays

The detection of CWD prions in tissues via immunohistochemistry (IHC) relies on the binding of antibodies against the prion protein (Guiroy et al., 1991). This assay has long been the official test, in many respects the "gold standard" (Box 4.1) for postmortem detection of CWD prions in the obex and specific lymph nodes in the neck region (retropharyngeal lymph nodes, RPLN) for regulatory testing purposes. This approach requires examination and confirmation by a trained pathologist. Because amyloid accumulates in these tissues over time, IHC results have been reported as a binary (positive/negative) or as a qualitative estimate of disease stage (e.g., Miller and Williams, 2002; Spraker et al., 2002b; Spraker et al., 2004; Keane et al., 2008a; Fox et al., 2006; Thompson et al., 2012; Haley et al., 2016a; Haley et al., 2016b; Spraker et al., 2015; also see Chapter 2). The use of acid treatment or protease digestion techniques to effectively degrade the normal prion protein ensures detection of the disease-associated prions (Guiroy et al., 1991), although these techniques have limited ability to detect forms of PrP^{Sc} that are sensitive to protease that may occur in some infections (Safar et al., 2005). As a result, despite its high specificity for detecting PrP^{CWD}, IHC sensitivity is lower than both bioassay and amplification assays (Haley et al., 2012; Haley et al., 2009a), further discussed below.

The commercial Bio-Rad CWD ELISA test kit is an official test for CWD when used according to USDA program standards (USDA-APHIS, 2019). ELISA is reasonably fast and can handle many samples at a time. It is considered more sensitive than western blot and IHC and can be used for testing lymphoid/neural samples beyond retropharyngeal lymph nodes and obex, for CWD (e.g., tonsil and recto-anal mucosa associated lymphoid tissue, RAMALT, Haley and Richt, 2017). Like IHC, ELISA may not be able to detect low concentrations of PrP^{Sc} (McNulty et al., 2019).

Although rarely used, western blotting is another diagnostic method dependent on antibody-antigen interactions, but unlike IHC or ELISA, detection depends on finding CWD PrPSc in partially purified tissues passed through a gel matrix (Guiroy et al., 1993). This technique separates proteins based on mass and allows direct visualization of different sized protein bands, an essential feature required for the identification and discrimination of different prion strains. Because western blotting, like IHC, relies on separating abnormal (PrPSc) from normal (PrPC) prion through enzymatic (protease) digestion, it cannot detect CWD prions that are less resistant to the enzyme and so the test may have limited value in detecting low concentrations of prions that may be present in early, nonclinical stages of infection (see Table 4.2).

In Vitro Amplification Assays

Over the past two decades, two distinct pathways for *in vitro* prion amplification have been developed. These pathways largely mirror the development of nucleic acid amplification assays for the detection of other infectious agents, beginning with a qualitative assay (PMCA) first described in 2005 that is similar to polymerase chain reaction (PCR) detection of nucleic acids (Saá, Castilla, and Soto, 2005). In 2007, a more quantitative assay (RT-QuIC) with utility comparable to real-time, quantitative PCR was developed (Atarashi et al., 2007). Although they have been widely used experimentally, in some cases undergoing extensive validation, neither have yet been approved for use in regulatory or conventional diagnostic settings. Their potential utility in disease scoring approaches has not yet been explored. A summary of these tests, including their advantages and disadvantages, is found in Table 4.3.

The PMCA assay involves the cyclical conversion of PrP^{C}, typically derived from transgenic (genetically modified) mouse brain PrP^{Sc}. This process results in new protein strands that are then fragmented and promote further conversion from normal to abnormal prion protein (Morales et al., 2012; Soto, Saborio, and Anderes, 2002). As such, PMCA is a highly sensitive assay, as it can amplify the equivalent of a single molecule of PrP^{Sc} (Saá, Castilla, and Soto, 2006), and it is well suited to detect prion infection in early, preclinical stages of disease. PMCA has been used for the detection of prions in

TABLE 4.3 In vitro amplification tests for CWD

Assay	Strengths	Weaknesses
PMCA	• Mouse-derived PrP^{C} may more accurately mimic natural prion molecular alterations. • PMCA products maintain the infectious properties of the PrP^{Sc} input, adding a strain-specific diagnostic value. • PMCA products can be used to study infectious mechanisms. • Evaluation of host range of specific prion strains, and zoonotic potentials. • Suitable modifications to allow testing large numbers of samples.	• Qualitative (but adaptable for partial quantitation) • Generation of infectious prions (impractical in diagnostic settings) • Depends on proteinase digestion for visualization of PrP^{Sc}. • Extended assays times compared with RT-QuIC
RT-QuIC	• Relatively rapid (24-48hrs) • Quantitative • Bacterial-produced, recombinant PrP^{C} substrate. • Does not generate infectious PrP^{Sc}, thus ideal for diagnostic settings. • Does not require enzyme pre-treatment for PrP^{Sc} visualization. • Easily adapted for large numbers of samples	• May need sample pre-treatment at low levels of PrPSc • Can not currently distinguish CWD strains. • Does not allow separate identification of prion species of origin. • Products are not suitable for characterizing prion strain properties.

SOURCE: Committee generated.

different sample types, including biological (Castilla, Saá, and Soto, 2005; Chen et al., 2010; Gonzalez-Romero et al., 2008; Haley et al., 2012; Park et al., 2018; Saá, Castilla, and Soto, 2006) and environmental samples (Nagaoka et al., 2010; Ness et al., 2022; Nichols et al., 2009). Relevant to CWD, PMCA has shown the potential for evaluating large numbers of samples and may be superior to both IHC and ELISA for screening RPLN samples (Benavente et al., 2023), although these findings were not confirmed through secondary testing (e.g., mouse bioassay or inter-laboratory validation). PMCA has also been used to detect prions in unearthed deer carcasses (Soto et al., 2023d), and at sites where taxidermal processing of infected carcasses was suspected (Soto et al., 2023a). The advantages of PMCA include the amplification of infectious prions from a sample while maintaining strain characteristics and species specificity (Castilla, Saá, and Soto, 2005; Deleault et al., 2007; Wang et al., 2010). Because of its dependence on western blotting as a read out, PMCA offers another advantage through the visualization of PrP^{Sc}, allowing for strain typing. Although newer methods using a simplified approach to western blotting known as "dot blotting" have been described (Benavente et al., 2023), PMCA can take 3-21 days for prion detection, requires both transgenic mice and specialized equipment, and results in an amplified infectious product (Atarashi et al., 2008; Morales et al., 2012).

Like PMCA, RT-QuIC is an *in vitro* amplification assay that depends on the seeded conversion of normal prion protein, in this case produced by bacteria (i.e., "recombinant" protein), to misfolded PrP^{Sc}. Amyloid formation is detected in real time with the insertion of a fluorescent molecule (thioflavin T) in the newly formed protein aggregates (Atarashi et al., 2008; Orrù et al., 2017). RT-QuIC is highly sensitive—capable of detecting approximately 1 femtogram (i.e., 10^{-15} grams), well below the dose necessary to induce an infection (See Chapter 2) of misfolded PrP^{Sc} (Atarashi et al., 2011). RT-QuIC has greater sensitivity in the detection of CWD prions than immunoreactive diagnostic tests (i.e., IHC and ELISA), particularly in earlier stages of infection (Haley et al., 2020a; Henderson et al., 2020; Manne et al., 2017; McNulty et al., 2019; Picasso-Risso et al., 2022). Multiple reports suggest that both RT-QuIC and PMCA have similar detection limits when brain extracts from infected animals are used (Hoover et al., 2016; McNulty et al., 2019; Kramm et al., 2017). The advantages of RT-QuIC broadly include its short turnaround time, quantitative abilities, high throughput potential, high degree of cross-laboratory correlation, and sensitivity using otherwise challenging biological and environmental samples (Table 4.3

and Haley et al., 2020a; 2020b; Burgener et al., 2022; Schwabenlander et al., 2024; and Huang et al., 2024, among others).

RT-QuIC, like PMCA, has the potential to provide strain discrimination, although it has not yet been used to differentiate CWD strains specifically (Masujin et al., 2016; Levavasseur et al., 2017; Orrù et al., 2015). A key advantage of RT-QuIC, separating it from both immunoreactive assays and PMCA, is that the procedure does not require an enzyme digestion step, thus allowing for the additional detection of protease-sensitive forms or strains of the CWD prion. Importantly, the product of the assay is not infectious, an important feature in diagnostic settings. Like PMCA, RT-QuIC requires specialized laboratory equipment and trained personnel. Early research suggests a high level of diagnostic agreement between RT-QuIC and ELISA (99.5%) and IHC (99.7%) (Holz et al. 2022). Given that RT-QuIC presently is used almost exclusively in research settings, current protocols differ in specific amplification conditions, reaction thresholds for designating positive or negative results, and data analysis (Rowden et al., 2023). Moving RT-QuIC into a diagnostic realm requires standardization of the protocol for different tissues and sample types to ensure confidence in test performance, reproducibility, and repeatability. Appropriate training, use of standardized positive and negative controls, and the inclusion of multiple replicates for each test sample is important to decrease or eliminate the appearance of false positive or false negative results.

NEXT GENERATION DETECTION ASSAYS

A new generation of diagnostic assays have emerged in response to the need for enhanced detection sensitivity in samples with ultra-low prion concentrations—especially in samples collected antemortem—and in response to the need for field-deployable assays for more rapid turn-around of test results. Newer enhancements to the RT-QuIC procedure, for example, including the addition of magnetic beads (Denkers et al., 2016; Henderson et al., 2015) and silica nanoparticles (Christenson et al., 2023) show promise for increasing detection sensitivity in antemortem sample types. These modifications leverage prion-binding characteristics to overcome assay interference by inhibitors and increase the efficiency of amyloid protein formation, thereby overcoming previous challenges in detection related to low prion concentrations in clinically accessible samples. Field-deployable assays, such as a microfluidic microelectromechanical biosensor, which utilize positive dielectrophoresis and monoclonal antibodies attached to electrodes in a microfluidic chamber to concentrate, trap, and detect prions, have also been developed as low-cost, sensitive diagnostic options (Muhsin et al., 2023). Early experiments demonstrate that this biosensor is 10 times more sensitive than ELISA at detecting PrP^{Sc} and does not require pretreatment with proteolytic enzymes to prevent cross-reaction with PrP^{C} (Muhsin et al., 2023). MN-QuIC is also described as a field-deployable diagnostic option, which integrates QuIC methods for prion amplification with the binding characteristics of misfolded prions to gold nanoparticles for prion detection based on color change that can be visually identified or measured by light-absorbance (Christenson et al., 2022). This low-cost and portable option was recently tested in the field and found to have a sensitivity of 95.7% and specificity of 100%, although validation in larger cohorts and in other laboratories are still needed (Christenson et al., 2022).

While these new diagnostic assays show high analytical sensitivity for the detection of PrP^{Sc} (see the earlier discussion in this chapter of sensitivity and specificity for an explanation of analytical versus epidemiological sensitivity), further research (e.g., bioassay confirmation) is needed to appreciate whether these extremely low quantities of detected prion are always associated with disease development and transmission. Likewise, additional research is needed to validate these assays for their use in the detection and surveillance of CWD for regulatory purposes, including estimates of test sensitivity and specificity across species, genotypes, and stages of disease, as well as repeatability and reproducibility across laboratories (USDA-APHIS, 2019).

POSTMORTEM VERSUS ANTEMORTEM TESTING

As noted above, current official CWD diagnostic protocols rely on conventional or standard methods of testing of tissues collected postmortem (ELISA, IHC), specifically the obex region of the brainstem and retropharyngeal lymph node (RPLN). With the caveat that these assays may not have perfect sensitivity, the utility of these approaches has been primarily to provide an estimate of disease prevalence at the farm, county/hunt unit, regional, or state levels, including the initial detection of disease incursion into new areas. There is limited official approval of these assays for antemortem testing—at least in part because the sensitivity of clinically-accessible tissues available for antemortem testing is lower than that of those collected postmortem (Haley et al., 2016; Thomsen et al., 2012). Provisionally, some agencies have begun using biopsies of RPLN, tonsil or RAMALT to screen and monitor herds with known exposure histories[3] (Monello et al., 2014). Research efforts to validate additional clinically accessible samples for antemortem CWD testing using amplification assays have been underway (see Appendix D), among them are RT-QuIC testing of ear pinna biopsy (Ferreira et al., 2021; Burgener et al., 2022). Box 4.2 describes hypothetical applications of this method for antemortem testing in combination with provisionally approved tissue testing (e.g., RAMALT and tonsil). While these early studies are promising, limited study numbers and small sample sizes justify further research on this front. Because such methodologies can involve the use of non-disposable sampling equipment (e.g., tonsil biopsy), which has the potential to become contaminated with infectious prions (Rutala and Weber, 2010; Secker, Hervé and Keevil, 2011, Laurenson, Whyte and Fox, 2001), and the introduction of minor oral lesions can facilitate CWD transmission (Denkers, Telling, and Hoover, 2010), more attention is needed related to the implementation of appropriate biosecurity practices when such techniques are employed.

BOX 4.2
Quantifying Uncertainty in CWD Prion Detection Using Surveillance Strategies

There is precedence for using combinations of diagnostic tests in disease control programs to overcome the limitations of individual test methods (e.g., the APHIS Bovine Tuberculosis Eradication Program [USDA, 2005]). This hypothetical example demonstrates how overall sensitivity (Se) and specificity (Sp) of CWD prion detection may be estimated when different test methods may be combined for antemortem CWD prion detection (see Appendix D for Se and Sp estimates of individual tests). Specifically, RT-QuIC applied to ear punch biopsies, RAMALT and tonsil biopsies or immunohistochemistry (IHC) of RAMALT and tonsil biopsies were examined, as they might be used for live-animal testing in series or in parallel. For tests used in series of each other, we estimated Se and Sp as:

$$Se = Se1 * Se2,$$
$$Sp = Sp1 + Sp2 - (Sp1 * Sp2),$$

where *Se1* and *Sp1* represent published Se and Sp estimates for Test 1 and *Se2* and *Sp2* represent published Se and Sp estimates for Test 2, respectively (Dohoo, Martin, and Stryhn 2014). For tests used in parallel, we estimate Se and Sp as:

$$Se = Se1 + Se2 - (Se1 * Se2),$$
$$Sp = Sp1 * Sp2$$

(Dohoo, Martin and Stryhn 2014). All Se and Sp point estimates used in these calculations were obtained from published studies (Table 4-2-1).

continued

[3] See https://www.tahc.texas.gov/animal_health/elk-deer/PDF/TAHCCertifiedCWDPostmortemSampleCollectionRecertification.pdf) (accessed July 25, 2024).

BOX 4.2 *continued*

TABLE 4-2-1 Breakdown of Testing Strategies Implemented Across 8 Different Scenarios of Application

Test	Strategy	Worst-case Se/Sp	Best-case Se/Sp
Test 1	Ear biopsy by RT-QuIC	0.81/0.91[a]	0.95/1[b]
Test 2	2nd Ear biopsy by RT-QuIC	0.81/0.91[a]	0.95/1[b]
Test 3	RAMALT by RT-QuIC or IHC	0.7/0.94 (RT-QuIC)[c]	0.80/0.99 (IHC)[d]
Test 4	Tonsil biopsy by RT-QuIC or IHC	0.89/0.97 (RT-QuIC)[e]	0.99/1 (IHC)[f]

Se: Estimated test sensitivity; Sp: Estimated test specificity.
[a] Estimated in white-tailed and mule deer by Ferreira and others (2021).
[b] Estimated in white-tailed deer by Burgener and others (2022).
[c] Estimated in white-tailed deer by Haley and others (2016a).
[d] Estimated in white-tailed deer by Keane and others (2009) and Thomsen and others (2012).
[e] Estimated in white-tailed deer by Picasso-Rizzo and others (2022).
[f] Estimated in mule deer by Spraker and others (2002b).

Approach: Overall, Se and Sp of CWD prion detection was estimated under 8 different scenarios (Table 4-2-2). In all scenarios, initial screening by RT-QuIC of an ear biopsy is assumed (Test 1), and if positive, would be followed by two confirmatory tests (Test 2 and either Test 3 or 4) used in parallel (with either being positive being a confirmatory result). In scenarios 1-3, a worst-case scenario was assumed using the lowest Se and Sp point estimates published for RT-QuIC testing of ear biopsies (Table 4-2-1). Scenarios 4-6 are a best-case scenario where the highest Se and Sp point estimates published for RT-QuIC of ear biopsies and highest Sp estimates of confirmatory tests were used. In scenarios 1 and 4, if Test 1 ear biopsy is positive, the animal is assumed to be retested by RT-QuIC of both ear biopsy and RAMALT. In scenarios 2 and 5, the secondary tests are assumed to include RT-QuIC of ear biopsy and IHC of RAMALT. For scenarios 3 and 6, confirmatory testing is assumed to be by IHC of RAMALT alone. Finally, in scenarios 7 and 8, secondary testing is assumed to be by RT-QuIC testing of a second ear biopsy and RT-QuIC (scenario 7) or IHC (scenario 8) of tonsil biopsy.

Outcomes: The lowest Se estimates were observed when only IHC of RAMALT was used as a secondary, confirmatory test (Table 4-2-2, Scenarios 3 and 6). In contrast, confirmatory testing that included repeat testing of ear biopsy by RT-QuIC in parallel with either RT-QuIC or IHC of RAMALT increases Se to 76-78% assuming the worst-case scenario (Table 4-2-2, Scenarios 1 and 2) or 94% Se assuming best-case scenario Se and Sp estimates (Table 4-2-2, Scenarios 4 and 5). Se estimates were highest when secondary testing included tonsillar biopsy (Scenarios 7 and 8). Under all scenarios, the Sp was 99-100%, meaning the probability that deer uninfected with CWD will test positive (i.e., false positive diagnosis) under any of these antemortem testing scenarios was 1% or less.

TABLE 4-2-2 Estimates of Overall Sensitivity and Specificity When Different Tissues and Testing Platforms are Used in Series and Parallel

	In series				
		In parallel		Se	Sp
Scenario 1	Test 1	Test 2	Test 3 (RTQ)	76%	99%
Scenario 2	Test 1	Test 2	Test 3 (IHC)	78%	99%
Scenario 7	Test 1	Test 2	Test 4 (RTQ)	79%	99%
Scenario 3	Test 1	Test 3 (IHC)		65%	100%
Scenario 4	Test 1	Test 2	Test 3 (RTQ)	94%	100%
Scenario 5	Test 1	Test 2	Test 3 (IHC)	94%	100%
Scenario 8	Test 1	Test 2	Test 4 (IHC)	95%	100%
Scenario 6	Test 1	Test 3 (IHC)		76%	100%

NOTE: Darker rows are estimates calculated using the lowest sensitivity and specificity estimates for RT-QuIC of ear biopsies, otherwise highest estimates of sensitivity and specificity for RT-QuIC of ear biopsies were used (lighter rows).
Se: Estimated test sensitivity; Sp: Estimated test specificity; Test 1: RT-QuIC of ear biopsy; Test 2: RT-QuIC of ear biopsy; Test 3: RT-QuIC or IHC (as specified in the table) of RAMALT; Test 4: RT-QuIC or IHC (as specified in the table) of tonsil.

As it has for other notifiable diseases like tuberculosis and brucellosis, antemortem testing could in theory be used prior to the transport of captive cervids recognizing that some animals in earlier stages of infection may go undetected. Past studies focused on antemortem testing have also examined the role of test and cull strategies in lowering disease prevalence in free-ranging and semi-free-ranging cervids, with mixed success (Haley et al., 2020a; Wolfe et al., 2018). The utility of antemortem testing, therefore, has primarily been applied to herd-level screening where concerns regarding imperfect test sensitivity may be balanced by high testing rates and not individual animal disease status determination. However, even with these recognized limitations, antemortem testing and surveillance—particularly in the management of CWD in captive herds—is recognized as critical to enhancing CWD control, monitoring the effectiveness of prevention in reducing the risk of CWD spread through animal movement, improving animal welfare, and reducing the economic impacts of CWD on herd owners (Henderson et al. 2013, Boden et al., 2010, Burgener et al., 2022). Despite advantages, antemortem testing use is not expected to supplant ongoing postmortem surveillance or confirmatory testing through the USDA CWD Herd Certification Program (HCP).

EARLY PHASE AND PRECLINICAL FALSE NEGATIVES

Key aspects of CWD pathogenesis likely affect the ability to detect misfolded prions in samples collected either ante- or postmortem and may influence detection through both conventional diagnostic approaches as well as experimental amplification assays. Importantly, the slow accumulation of prions in brain and peripheral tissues and fluids limits test utility to tissues with a sufficient prion burden at that stage of disease. This is highlighted by the absence of detectable prions, through IHC and ELISA, in postmortem obex collections from white-tailed deer in very early stages of disease—in these cases, prions may only be identified in RPLN tissues. The phenomenon may also be seen through the reduced sensitivity of both RAMALT and nasal brushings in earlier disease stages. Although past investigations suggest that experimental amplification assays may improve sensitivity in these cases (Haley et al., 2020a; Haley et al., 2016a), there will inevitably be periods of early infection where prion burden remains below the threshold of detection (Henderson et al., 2020). Future studies may highlight additional tissues (e.g., gastric-associated lymphoid tissue (GALT) or enteric nervous system components) involved in the initial stages of pathogenesis, which may enhance test sensitivity in earlier disease stages.

Either alone or in concert with disease progression, test sensitivity may also be reduced in animals with less-susceptible *PRNP* alleles. Antemortem testing sensitivity, using RAMALT for example, is reduced in deer and elk carrying the 96S or 132L alleles, respectively (Haley et al., 2016a; Haley et al., 2016b; Thomsen et al., 2012). This may be because animals with these alleles are generally in earlier stages of disease than those with wild-type 96G or 132M alleles (Haley et al., 2016a; Haley et al., 2016b). It remains to be shown whether *PRNP* genotype modulates test sensitivity in the field, and whether amplification assays may likewise suffer from this potential limitation.

BIASES IN SAMPLING AND TESTING

Limitations in sampling strategy, diagnostic test methodology, and implementation in CWD surveillance can introduce bias and impact understanding of the epidemiology of CWD (see Box 4.3 for some logistical issues associated with postmortem sampling). For example, postmortem surveillance for CWD utilizing the detection of PrP^{Sc} in RPLN has become a standard of practice for many state-level hunter-based surveillance programs because of its sensitivity for early detection in subclinical cases. However, this sampling strategy would likely misdiagnose CWD phenotypes where PrP^{Sc} is unlikely to be present in peripheral lymphoid tissues (see Chapter 2; Benestad et al., 2008; Güere et al., 2022). Thus, paired sampling of the obex to distinguish these sporadic phenotypes from the contagious lymphatic phenotypes of CWD is critical to understanding the epidemiology and risk among captive herds as well as in new geographic areas where CWD has been detected in free-ranging populations. However, even among prion strains typically found across North America, the stage of disease (or time since infection)

and genetics (that influence progression of disease) can influence prion detection by the well-accepted assays (described earlier in this chapter). Further, CWD pathogenesis in deer and elk are distinct: in deer, PrP^{Sc} accumulation occurs earlier in the RPLN than the obex; but in elk, PrP^{Sc} accumulation in the obex may occur earlier. Given these species variations, routine sampling and testing of RPLN only could introduce bias when it comes to CWD surveillance among elk, where individuals in earlier stages of disease would likely be under detected. When it comes to testing, the use of protease or acid treatments in some assays to eliminate PrP^{C} would also result in the removal of CWD strains less resistant to protease digestion (Gambetti et al., 2008; Head et al., 2009; Benestad et al., 2003; Orge et al., 2004; Klingeborn et al., 2006, Duque Velasquez et al., 2020). Thus, any cases caused by protease-sensitive strains of CWD would be missed by the routine use of IHC and ELISA in surveillance.

BOX 4.3
Postmortem Sampling Logistics

Sampling cervids for CWD using USDA-approved diagnostic methods generally requires postmortem sampling of specific tissues by trained individuals. Official testing in captive herds (see Chapter 6) is required to be conducted in USDA approved laboratories that are certified as part of the National Animal Health Laboratory Network. Coordination between cervid-owners or hunters and local officials is often necessary to obtain samples. For example, sampling done in conjunction with hunter harvest-based surveillance by states or Tribes often requires that hunters bring carcasses or the severed heads to designated locations for trained officials to extract appropriate tissues (see Figure 4-3-1). The sample or animal head is then sent to an approved diagnostic laboratory where tissues are removed for testing (see Figure 4-3-b). Tissue sampling must also be accompanied by appropriate metadata (e.g., location of harvest, sex and age class of the animal) for utility in population-level surveillance. Time lags in testing may impact hunters' ability (particularly subsistence or Amish hunters) to make butchering and consumption decisions.

FIGURE 4-3-1 Elk head deposit bin. Hunters are asked to sever and deliver the heads of their animals to specific drop-off locations.
SOURCE: Shutterstock, ID 1577197171.

FIGURE 4-3-2 The heads are collected and prepared for sampling in the laboratory.
SOURCE: Alberta Fish and Wildlife.

LIMITATIONS AND PROMISING DIRECTIONS IN EARLY CWD PRION DETECTION AND CONTROL

Most CWD testing is conducted with ELISA or IHC methods in 32 National Animal Health Laboratory Network (NAHLN) laboratories[4] to screen tissues collected postmortem. The recent advances

[4] See https://www.aphis.usda.gov/labs/nahln/approved-labs/cwd (accessed August 24, 2024).

in prion amplification assays (e.g., PMCA, RT-QuIC) prompt the question of whether the findings of small-scale studies of assay validation (see Appendix D) truly reflect enhanced sensitivities, and ultimately whether they may be used by NAHLN laboratories. Conclusively answering questions regarding these issues requires either (1) inter-assay comparisons that rely on a commonly accepted reference standard (e.g., mouse bioassay, which may overlook true positive cases that are either very early in the pre-clinical stages or where prion burden is below the level of detection), or (2) longitudinal studies using antemortem sampling and eventual postmortem confirmation using conventional testing methods. In the first case, the resources currently required for bioassay confirmation of samples evaluated by both conventional and amplification assays make this approach impractical, at least on the scale necessary to permit reevaluation of the current acceptance of ELISA and immunohistochemistry as diagnostic reference standards. Small-scale studies have successfully employed bioassay confirmation of infection where postmortem testing has resulted in IHC-negative, PMCA-positive white-tailed deer cases (Haley et al., 2009a). In the second case, the lack of regulatory approval or validation of any antemortem testing approaches in cervids makes longitudinal, live animal testing approaches problematic. A three-year longitudinal study in elk, however, found that animals with IHC-negative, RT-QuIC-positive RAMALT biopsies would eventually become IHC-positive either ante- or postmortem, or would be lost to follow-up in the field and presumed dead. Improvements in sensitivity provided by amplification techniques in this case were estimated to be 30% or more over IHC (Haley et al., 2020a). An adjunct approach to these two scenarios is the employment of statistical methods that integrate the uncertainty resulting from the absence of a true gold standard (see Box 4.1) of comparison (Picasso-Risso et al., 2022; Wyckoff et al., 2015). This approach recognizes the inherent imperfection of all diagnostic assays in the discrimination of true disease status (i.e., infection), and rather than ignoring those limitations, incorporates our current understanding of those limitations into the estimation of more accurate measures of test performance. Although presently available data highly suggest that amplification assays do afford improved sensitivity over conventional approaches, broader studies in the future which incorporate bioassay as a reference standard may provide irrefutable data on the true sensitivity and specificity of both conventional and amplification assays.

A major limitation to the use of antemortem testing in captive herd management is the relative lack of official USDA antemortem sampling and testing as part of the HCP, although some states have implemented their own antemortem testing protocols. Currently, USDA-APHIS only approves the testing of RAMALT or RPLN biopsies by IHC under certain conditions for the antemortem detection of CWD in captive white-tailed deer (i.e., not elk, mule deer, or other cervid species) (USDA-APHIS, 2019). These conditions are that (1) at least two whole herd serial tests are performed when RAMALT biopsies are screened; (2) a whole herd is tested (rather than an individual tested prior to movement); and (3) the genotype at codon 96 must be known for all individuals in the herd and more than 50 percent of the herd must have the 96G/96G genotype (USDA-APHIS, 2019). Importantly, this antemortem testing strategy may only be applied in cases where a herd has been identified as CWD-exposed (i.e., through trace-back) or otherwise epidemiologically linked to a CWD-positive herd. The application is not approved for use in routine surveillance.

The requirements described above result in limitations that need to be overcome to manage CWD more effectively. First, because of the natural pathogenesis of CWD in cervids (Henderson et al., 2020; Haley and Richt, 2017; see Chapter 2) the sensitivity of RAMALT, a more accessible tissue sample than RPLN biopsy, is comparably lower (estimated 70-85% sensitivity; e.g., Haley et al., 2016a; Haley et al., 2016b; Tewari, et al., 2022). Secondly, the identification of PrP^{CWD} in tonsils, which is more accessible than RPLN, while also requiring specialized, single-use equipment to avoid iatrogenic transmission (see previous section on Postmortem versus Antemortem Testing), allows for earlier identification of CWD in white-tailed deer (Henderson et al., 2020) than RAMALT. Lastly, prion detection by amplification assays like RT-QuIC and PMCA has been shown to improve upon the sensitivity of IHC (Benavente et al., 2017; Haley et al. 2020 [https://pubmed.ncbi.nlm.nih.gov/32033521/]), leading to earlier detection capabilities in some cases (Henderson et al., 2020). USDA-APHIS has initiated efforts to validate RT-QuIC as an official antemortem test of RAMALT and RPLN but progress toward validation and approval is

uncertain. Assuming that appropriate biosecurity methods are adopted and employed to prevent iatrogenic transmission during sampling, these alternative sampling approaches may provide avenues for validation studies to enhance herd surveillance. Further, because the HCP conditions for antemortem testing restricts its use in routine surveillance, there is limited opportunity to utilize antemortem surveillance for early detection and control (e.g., premovement), resulting in the potential for missed management opportunities.

As new antemortem diagnostic testing capabilities emerge, efficient epidemiological validation of sampling and testing of cervids is needed. Cooperation between USDA-APHIS and the research community is critical such that requisite study designs for official test validation can be implemented early and by more research teams. This would involve transparency related to the criteria required for official test validation, collaboration, and communication between those reviewing and approving protocol validation data for official purposes and researchers developing these new technologies. Test validation and assessment of performance is an ongoing process, which needs to continue even after approval and implementation. Early validation studies cannot encompass and assess test performance under all conditions that characterize the natural settings in which they will be used. The impact of repeated sampling and tissue regeneration (e.g., RAMALT, tonsil, ear biopsy, etc.; e.g., Geremia et al., 2015; Monello et al. 2014) on prion detection also needs to be evaluated in ongoing studies. However, longitudinal studies that support such validation efforts are difficult to perform given timelines of current funding mechanisms (e.g., through the CWD Cooperative Agreement Funding Opportunities through USDA-APHIS; USDA Animal and Plant Health Inspection Service, 2024). Strategies for implementing antemortem testing into routine surveillance for early CWD prion detection would benefit ongoing research efforts into diagnostic test assessment, validation, and robustness. Knowledge could be advanced with the recognition that all tests are imperfect (Dohoo, Martin, and Stryhn 2014; see Appendix D, Box 4.4, that uncertainty and animal welfare are integrated into response plans, and that interested parties (e.g., herd owners, hunters) could be enlisted to enhance opportunities for sample and data collection.

BOX 4.4
Interlaboratory Agreement

A metric used in the diagnostic validation of assays is the assessment of interlaboratory agreement. This process examines how well different laboratories, conducting the same testing procedures on the same samples, can obtain the same results (Dohoo, Martin, and Stryhn, 2014). While there are numerous studies examining the sensitivity and specificity of the newer amplification assays for CWD detection (see Appendix D), studies to evaluate interlaboratory agreement require more coordination and collaboration, and thus, are fewer in number. However, a recent study examining interlaboratory agreement of RT-QuIC for the detection of CWD in retropharyngeal lymph nodes from 50 white-tailed deer (with three different codon 96 genotypes) demonstrated high interlaboratory reproducibility, even with some variation in RT-QuIC protocols (Darish et al., 2024). The committee is aware of a similar interlaboratory study of RT-QuIC for CWD detection in RAMALT that demonstrated lower repeatability, particularly among animals in early stages of infection. The latter study is yet unpublished. To advance the diagnostic validation of newer CWD detection technologies, standardized protocols and additional research efforts such as these are needed to ensure test accuracy.

SURVEILLANCE

Surveillance has been foundational in attempts to detect and contain CWD in the United States and elsewhere (Miller et al., 2000; Samuel et al., 2003; Kahn et al., 2004; EFSA BIOHAZARD Panel, 2023; Thompson et al., 2023). The general principles of sampling (e.g., Cannon and Roe, 1982) as applied to CWD surveillance have been described in detail and periodically refined (e.g., Samuel et al., 2003; EFSA BIOHAZ Panel, 2023). The few reported examples of apparent containment of CWD in the

wild have been associated with outbreaks detected at very early stages, emphasizing the value of surveillance to control efforts (Fischer and Dunfee, 2022). Unfortunately, the effort required to detect foci where CWD has been newly introduced—especially in the wild—may be considerably greater than that required to monitor changes and trends after foci have been detected (EFSA BIOHAZ Panel, 2023). Yet from the data reported, it is evident that sampling efforts in many jurisdictions expanded only after CWD had been epidemiologically traced to or detected within or near the jurisdiction's boundaries, thereby delaying management responses and distorting the true timeline of CWD emergence across the United States (e.g., Thompson et al., 2023; Ruder, Fischer, and Miller, 2024; also see Chapter 5).

Detecting CWD in captive cervid facilities can be relatively straightforward from a technical standpoint but is inconsistently applied (e.g., some states require captive facilities to participate in CWD surveillance, others do not). Because CWD-infected cervids do not recover and are expected to die, screening appropriate postmortem tissue samples (e.g., RPLN ± brainstem at the obex) collected from all cervids that die of any cause in a facility using any of the approved diagnostic tests yields a high probability (greater than or equal to 0.95) of detecting infected cervids, although this approach risks disease establishment during expected incubation periods that may extend to several years after infection (e.g., O'Rourke et al., 2007; Miller et al., 2012). Compromises in the completeness of mortality screening (e.g., capping the number of submissions in the face of a natural disaster or hemorrhagic disease outbreak, allowing for some number of "missed" death losses, or exempting young cervids) may erode detection probability to varying degrees. Surveillance approaches emphasizing high-risk cervids including "fallen stock" (i.e., unexplained illnesses and deaths), natural mortalities, and cervids showing signs of clinical disease suggestive of CWD also offer a high probability of eventual detection (Miller et al., 1998; Samuel et al., 2003; Walsh, 2012; EFSA BIOHAZ Panel, 2018; EFSA BIOHAZARD Panel, 2023) but may miss some individuals in early stages of disease that die but are not considered high-risk (e.g., apparently healthy cervids that are slaughtered or hunted). Whole-herd testing of live cervids has been proposed and occasionally used as an adjunct to mortality-based screening (Texas Animal Health Commission, 2022; M.W. Miller, personal communication, April 26, 2024).

Similar CWD surveillance principles outlined for captive cervids could apply to free-ranging cervids. However, application is considerably more difficult because cervids in the wild range over relatively large areas and their illnesses and deaths are not observable to the same degree as captive conspecifics (i.e., cervids belonging to the same species). Captive cervids residing in enclosures with features that resemble wildland habitats may present similar challenges for surveillance (e.g., Haley et al., 2020a). Comprehensive screening of all mortalities is rarely feasible in the wild. Instead, surveillance for CWD in free-ranging populations relies on screening a sufficient number of samples to assure a high probability of detecting at least one case given a target prevalence (proportion of infected cervids in the population of interest). Screening of hunter-killed, road-killed, found dead, and "high-risk" cervids in various combinations have been used in efforts to detect CWD foci in the wild since the 1980s (Williams and Young, 1992; Samuel et al., 2003; Walsh, 2012; EFSA BIOHAZARD Panel, 2018; EFSA BIOHAZARD Panel, 2023). Weighting approaches (e.g., Walsh and Miller, 2010; Walsh, 2012; Jennelle et al., 2018; EFSA BIOHAZARD Panel, 2023) have been developed to allow use of samples from various combinations of risk and demographic classes in designing CWD surveys and interpreting their results. Weighting samples according to evidenced-based risks of detection (Jennelle et al., 2018; Walsh and Miller et al., 2010) leverages inherent bias that may occur when testing samples from different deer demographics or sources of mortality, reducing the total number of samples needed to achieve the same detection probability as that needed for randomized sampling.

Data and experience have demonstrated that subdividing large geographic areas (e.g., a state, in the context of this report) into biologically- or administratively defined "primary sampling units" will improve the sensitivity and timeliness of detecting emergent CWD foci in the wild (Samuel et al., 2003; Diefenbach, Rosenberry, and Boyd, 2004; Joly et al., 2009; EFSA BIOHAZARD Panel, 2018; EFSA BIOHAZARD Panel, 2023; Fischer and Dunfee, 2022). Risk-based assessments (e.g., Russell et al., 2015; Fischer and Dunfee, 2022) may help prioritize the geographic areas of greatest potential importance in statewide surveys, but a systematic surveillance approach ultimately may be needed given the difficulty in

obtaining a comprehensive understanding of all potential sources of introduction risk (EFSA BIOHAZARD Panel, 2023). Combining data from different risk groups and accumulating the needed number of samples over several consecutive years can improve the practicability of CWD surveillance in large jurisdictions or other circumstances where sampling opportunities are limited (e.g., Jennelle et al., 2018; EFSA BIOHAZARD Panel, 2023).

Recommended CWD surveillance approaches are well-established (e.g., Joly et al., 2009; Samuel et al., 2003; Walsh and Miller, 2010). Key considerations include the sample size targets needed to detect outbreaks at relatively low apparent prevalence (e.g., 1 percent or less), the appropriate subdivision of large jurisdictions into smaller sampling units so outbreaks are still relatively localized at the time of detection, and temporal considerations needed to assure meaningful inferences can be made from the resulting data (Joly et al., 2009; Samuel et al., 2003; Diefenbach, Rosenberry, and Boyd, 2004; Walsh and Miller, 2010). The recent EFSA BIOHAZARD Panel (2023) includes a detailed overview and surveillance "toolkit". Other detailed reviews of approaches and recommendations for detecting CWD in free-ranging cervids, literature citations, and examples of successful implementation are available (e.g., EFSA BIOHAZARD Panel, 2018; Fischer and Dunfee, 2022; EFSA BIOHAZARD Panel, 2023). Nonetheless, individual jurisdictions face a variety of limitations in execution of those approaches (Fischer and Dunfee, 2022; Thompson et al., 2023) and consequently the inferences regarding CWD occurrence that can be gained from surveillance have varied widely over time and among jurisdictions (e.g., Thompson et al., 2023; Ruder, Fischer, and Miller, 2024). See Chapter 6 for further discussion of surveillance in the context of disease prevention and control.

Assessing and Interpreting Surveillance Findings

Surveillance is conducted to inform CWD response and management by state, tribal, and federal agencies. Depending on the current state of CWD in a location (e.g., presumed absent, early introduction, high prevalence), surveillance goals, and therefore methodologies, may vary (see Chapter 6). Accordingly, there are several criteria by which surveillance systems might be assessed, including their sensitivity of case detection at specified levels of disease in a population (i.e., detection probability given an assumed prevalence value), specificity of detection (i.e., estimated potential for false alarms), cost-effectiveness and long-term sustainability in meeting agency goals. Ultimately, the veracity of reported "disease absence" (Box 4.5) and inferences about the timing of CWD introduction or emergence based on a "first detection" depend heavily on surveillance design (EFSA BIOHAZARD Panel, 2023; Ruder, Fischer, and Miller, 2024; also see Chapter 5). As discussed in the previous section, the number of cervids tested (sample size) and the size of the primary sampling or survey unit (i.e., the geographic area associated with the cervid population being sampled) together can influence the survey's sensitivity (i.e., the probability of detecting one or more cases in a population given an assumed prevalence value) with respect to early CWD detection in a population (Samuel et al., 2003; EFSA BIOHAZARD Panel, 2023).

Surveillance of Free-Ranging Herds

Detecting CWD outbreaks in free-ranging cervids at an early stage is desirable from a control standpoint (see Chapter 6, "Approaches for Controlling CWD in Free-ranging Cervid Populations") but is extremely difficult to achieve even with today's understanding and tools because early in an outbreak the number of cases and the size of the affected area are small (e.g., less than 10 infected cervids within less than 10 square miles) (Joly et al., 2009; EFSA BIOHAZ Panel, 2023). Surveys for CWD are typically designed to detect at least one case in a herd or population with 95 percent or 99 percent confidence where apparent infection prevalence (proportion of the number sampled testing positive) is greater than or equal to 1 percent (e.g., Samuel et al., 2003; Joly et al., 2009; EFSA, 2023). Achieving this high likelihood of detection requires screening approximately 300-500 harvested cervids from a herd or population of greater than or equal to 1,000 individuals (Cannon and Roe, 1982). However, inferences about negative outcomes of surveillance are constrained by the survey design. For example, finding no

cases among 300-500 samples collected from hunter-harvested cervids across an entire state provides reasonable certainty that CWD prevalence in the state's entire cervid population of perhaps a million or more individuals is no higher than 1percent (1 in 100) (Cannon and Roe, 1982; Samuel et al., 2003; Diefenbach, Rosenberry, and Boyd, 2004; EFSA BIOHAZARD Panel, 2023), but the finding offers little assurance that CWD is not present at lower statewide levels or in a more limited geographic distribution, as commonly encountered in the first several decades after its introduction (e.g., Joly et al., 2009; see below and Chapter 5 for further discussion). Diefenbach, Rosenberry, and Boyd (2004) illustrated an early application of these principles in considering approaches for statewide surveillance in Pennsylvania preceding CWD detection.

BOX 4.5
The (Confidence) Limits of "Zero" Detections

Reporting outcomes from CWD surveys in terms of whether cases were or were not detected has been commonplace for over two decades. Detecting zero cases from CWD surveillance is often perceived—and sometimes portrayed—as equating to the absence of disease despite obvious statistical limitations to such interpretations (Miller and Fischer, 2016; Ruder, Fischer, and Miller, 2024). This tendency aligns with a general observation that "… the occurrence of 'no events' seems to be viewed as very different both quantitatively and qualitatively from the occurrence of one or more events…" (Hanley and Lippman-Hand, 1983). Given these tendencies, accurate (re)interpretation and communication of "zero numerator" surveillance findings seem necessary elements of retrospective and future assessments of CWD's status nationwide.

The same statistical principles that apply to data from surveys with nonzero numerators (i.e., one or more cases detected) also apply to data with zero detections (Rümke, 1975; Cannon and Roe, 1982; Hanley and Lippman-Hand, 1983). Thus the "true" risk of CWD cases being present despite no detections in a random sample from a host population can be estimated with a set degree of confidence (typically 95%) as falling between zero and some upper limit. For example, a survey result of zero positives among 300 samples (n) screened (i.e., 0/300) offers 95% confidence (assuming perfect test sensitivity and specificity) that if CWD is present in the sampled population then the number infected is fewer than ~1 in 100 (upper 95% binomial confidence limit = 1.2%, which can be approximated as $3/n$ [Rümke, 1975; Hanley and Lippman-Hand, 1983]). For a population numbering ~10,000 individuals this would equate to the maximum number of cases falling in the range of 0-119. However, for a population of 1,000,000 individuals (and likely occupying a large geographic area), this same 0/300 result only confers 95% confidence that if present the number of cases would be ≤11,999.

The latter outcome illustrates the considerable degree of uncertainty about the absence of CWD in surveys of large host populations, thereby underscoring the need for designing surveillance at appropriate population/geographic scales (e.g., Samuel et al., 2003; Diefenbach, Rosenberry, and Boyd, 2004; Joly et al., 2009; EFSA BIOHAZ Panel, 2023) and communicating "zero numerator" outcomes in the context of the plausible upper limits of disease occurrence given "no detections to date".

Of note in the context of control, CWD outbreaks that have reached the levels of prevalence routinely targeted for detection at the geographic scales upon which surveys have been based may be well-established, thereby complicating prospects for effective control. For example, in a local population of approximately 10,000 cervids (e.g., occupying a county-sized area in a midwestern state), prevalence at greater than or equal to 1 percent would equate to already having 100 or more infected cervids present at the time of first detection; in a population with approximately 1,000,000 cervids (e.g., the population occupying an entire state), this could equate to having approximately 10,000 or more infected cervids present at the time of first detection. Detecting outbreaks at a lower prevalence (e.g., 1 in 1,000 or 0.1 percent) with equal confidence requires larger sample sizes (Figure 4.1; Cannon and Roe, 1982; Diefenbach, Rosenberry, and Boyd, 2004). For this reason, screening a few hundred samples across an entire state has at times failed to detect substantial but geographically localized CWD outbreaks

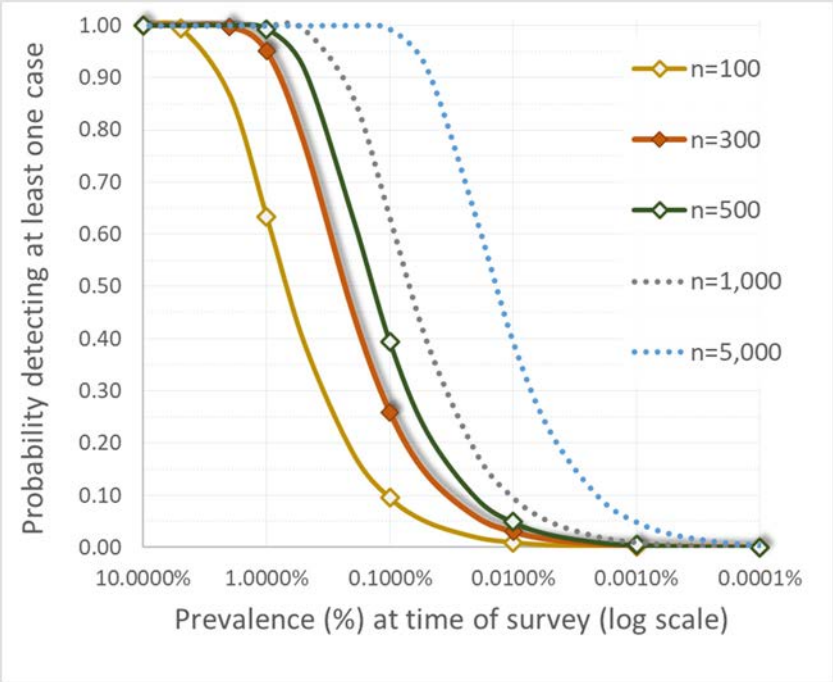


FIGURE 4.1 Relationships between the number of samples tested (*n*; lines) from a population of greater than or equal to 10,000 individuals, the survey design prevalence (assumed prevalence at the time of a survey; *x*-axis, expressed as percent and shown on a log scale), and the probability of detecting one or more cases in the survey sample (*y*-axis). To simplify comparisons, the points on each curve mark detection probabilities for respective sample sizes at 1%, 0.1%, 0.001%, and 0.0001%, as well as the design prevalence where each value of *n* approaches a detection probability of 1.0. The line representing the common sampling standard *n*=300 is emphasized for comparative purposes. Graph generated for this report using equations, assumptions, and additional context described elsewhere.
SOURCE: Cannon and Roe, 1982; Joly et al., 2009.

(Joly et al., 2004; Ruder, Fischer, and Miller, 2024). Surveys based on geographic areas the size of an entire state or larger have yielded misinformed assessments of CWD distribution and "absence" historically (EFSA BIOHAZARD Panel, 2018; Ruder, Fischer, and Miller, 2024; also see Box 4.5 and Chapter 5). It is for this reason that contemporary recommendations for designing CWD surveillance include subdividing large geographic areas (e.g., an entire state) into spatial subunits ("primary sample units") more equivalent in size to a wildlife management unit or county in order to assure reliable inferences can be drawn (Samuel et al., 2003; Diefenbach, Rosenberry, and Boyd, 2004; Joly et al., 2009; EFSA BIOHAZARD Panel, 2023).

Given current sample testing regimes which rely on highly specific tests (ELISA or IHC with greater than 99% specificity, see Appendix D for more detail on test performance) for CWD detection, it is unlikely that existing surveillance systems would experience false positive results (i.e., diagnosis of CWD in an uninfected animal). However, there are recognized biases in sampling design that could impact accuracy of the prevalence estimation. For instance, Conner and others (2000) demonstrated temporal trends in CWD prevalence across a harvest season, where a greater number of CWD cases were detected later in the harvest season. Thus, by estimating prevalence across all fall hunting seasons within the year (versus a single harvest season sample), these researchers were able to obtain a more accurate and unbiased CWD prevalence estimate. Differences in CWD risk across demographics can also bias prevalence estimates if sampling is not random and some demographics are under- or over-represented in a sample as compared to their distribution in the population. As noted in the previous section, however, demographic differences in risk can also be leveraged to maximize CWD detection while reducing

constraints on resources (Walsh and Miller, 2010; Jennelle et al., 2018). Similarly, it is important to be aware of potential spatial bias in harvest-based surveillance practices, considering both the nonrandom distribution of deer harvest by hunters as well as the nonrandom distribution of CWD-infected individuals across a landscape (Samuel et al., 2003; Diefenbach, Rosenberry, and Boyd, 2004; Farnsworth et al., 2006; Osnas et al., 2009). Finally, recognizing how sampling of vehicle-killed or hunter-harvested deer can over or underestimate detection probabilities or prevalence estimates is also critical (Nusser et al., 2008).

Agencies conducting CWD surveillance are challenged by the investment of resources needed for ongoing, active CWD surveillance (see Chapter 7). Because CWD surveillance is a resource intensive and seemingly perpetual endeavor, agencies need to weigh surveillance goals and the choice of sampling framework and sample size with the costs of the program. Most states and many Tribes conduct some level of CWD surveillance or monitoring each year through voluntary sample submissions from hunters; a smaller number require hunters to submit samples from cervids harvested in specific locations or years (e.g., annually, or on a rotation) to increase sample sizes and improve data precision.[5] Fischer and Dunfee (2022) provide several detailed examples of how state agencies have adapted their surveillance approaches based on changes in CWD epidemiology as well as the need for cost efficiency. An example of a network of midwestern tribal natural resource agencies coordinating CWD surveillance activities across Tribal lands is described in Chapter 6.

Surveillance of Captive Herds

Surveillance among captive herds has been led by the USDA HCP at the federal level and various state agencies (e.g., departments of agriculture, natural resource agencies, animal health boards) at the state level. However, implementation of captive herd surveillance through the HCP is inconsistent across states that permit captive cervid possession because it rests on voluntary participation by both states and captive herd owners (see further details in Chapter 6). At the time of writing this report, 28 states had approved HCPs (USDA, 2018). Also, because the federal program focuses surveillance efforts on the interstate movement of cervids, herds that only move cervids within a state may be missed by the federal surveillance program unless state agencies mandate broader participation in some form of herd certification or monitoring. Examining data from states where CWD monitoring has been a requirement of herd ownership may help determine the extent to which such surveillance has been effective in earlier CWD detection (as demonstrated by lower herd prevalence levels at time of detection) and reduced occurrence of multi-herd outbreaks related to captive cervid movements.

EARLY WARNING SYSTEMS – NEW HORIZONS IN SURVEILLANCE

Much surveillance is conducted among free-ranging cervids through sampling and testing of hunter-harvest or vehicle-killed animals or testing of unhealthy cervids that may be found dead or euthanized. In the captive cervid industry, participation in surveillance is voluntary in some states through participation in the USDA HCP whereas in other states participation in mortality-based CWD surveillance is a condition of cervid ownership.[6] There is currently no pre-diagnostic (e.g., syndromic or environmental) surveillance program for either captive or free-ranging cervid herds and populations. However, new surveillance technologies for the detection of prions in the environment or on contaminated surfaces (e.g., feeders; Yuan et al., 2022; Soto et al., 2023d; Huang et al., 2024) are being researched and may offer new opportunities for noninvasive early detection. For example, research is emerging to evaluate the efficacy of detecting CWD prions in environmental samples collected from white-tailed deer scrape sites. These are locations on the landscape where deer scrape away leaves and debris to expose bare soil and where they interact with overhanging branches for communication via scent

[5] See https://cwd-info.org/cwd-hunting-regulations-map/ (accessed May 7, 2024).
[6] See https://www.michigan.gov/dnr/managing-resources/wildlife/wildlife-disease/disease-monitoring/cwd/cwd-hunting-regulations/cwd-and-cervidae-regulations-in-north-america (accessed July 25, 2024).

marking, and they can be visited and used by many deer during a breeding season (Egan et al., 2023). Preliminary results demonstrate that PrP^{Sc} can be detected by RT-QuIC from soil and tree branch samples from scrape sites (Huang et al., 2024), offering another option for surveillance of CWD (Lichtenberg et al., 2023). Another early-detection approach being evaluated is the use of environmental swab sentinels for the detection of CWD prions at feed troughs and other surfaces on captive cervid facilities using RT-QuIC (Yuan et al., 2022; Huang et al., 2024) or PMCA (Soto et al., 2023d). These methods leverage the binding capacity of PrP^{Sc} to certain surfaces and the ability to extract these environmental prion proteins using swabbing technology (Yuan et al., 2022) for detection. Early findings demonstrate that the number of RT-QuIC-positive swabs in a herd pen correlates with CWD pen prevalence as determined by IHC (Schwabenlander et al., 2023a). The detection of prions in the environment using these methods has also been investigated for surveillance for environmental prion proteins at bait sites used during culling activities of free-ranging deer. Further work is needed, however, to determine how frequency of environmental prion detection on swabs or load in RT-QuIC correlates with the number of CWD-positive deer visiting bait sites (Schwabenlander et al., 2023a). Collectively, these unpublished preliminary findings show promise for their application in surveillance in ways that may enhance surveillance for CWD detection in a way that does not compromise animal welfare. Leveraging the capabilities of PMCA and RT-QuIC in pre-diagnostic sample testing and integrating these approaches with existing surveillance systems to confirm early CWD signals could provide a robust and powerful system for CWD detection, for informing management, and for ongoing studies into the ecology and epidemiology of CWD and its control on and off farm.

5
Epidemiology and Ecology of Chronic Wasting Disease

Chapter Highlights

- CWD outbreaks progress slowly, especially in the wild. As outbreaks grow, they can cause substantial numbers of cervid mortalities that can contribute to depressed growth or declines in cervid herds given sufficiently high prevalence.
- As of August 1, 2024, CWD has been reported in 35 states across the continental United States, affecting both captive and free-ranging cervid populations. It has persisted in most locations where detected and has shown a substantial increase in prevalence and considerable geographic expansion in some U.S. states over the last few decades.
- Natural and biological processes, human activities and behaviors, ecological influences, and host-prion dynamics all influence the spread of CWD. Knowledge and information about each of these factors and of their interrelationships have improved over time but remain incomplete. Ongoing and future control efforts could benefit from further applied research and studies to define and clarify contributors of spreading.
- Because chronic wasting disease outbreaks progress slowly, they can be difficult to detect in the earliest stages, especially in the wild. This makes the geographic spread of CWD difficult to understand and track in real time.
- Prions in the environment represent potential sources of exposures and infections. Information and data about how attractants, feeding sites, carcasses, feeds, farm equipment, captive cervid pens, and across-fence contacts contribute to CWD transmission are being studied.

Understanding how chronic wasting disease (CWD) outbreaks progress and how the disease spreads geographically at local and larger spatial scales has informed control strategies, just as diagnostics and surveillance have provided tools for detecting CWD and tracking trends (see Chapter 6 for discussion of surveillance and interventions). The European Food Safety's report on CWD summarizes decades of U.S. research on EFSA Panel on Biological Hazards (2019); see Figure 5.1. The factors that contribute to CWD spread, both positively and negatively, are complex and not well quantified in the literature. Issues such as climate (e.g., drought, severe winters), migration route interference, human land development, increased predation, and other diseases can affect the geography of CWD.

This chapter summarizes knowledge about the patterns and drivers of CWD spread and key features of its epidemiology and ecology. The chapter first discusses patterns of geographic growth and apparent spread at the regional and national scales. Drivers are then discussed, including anthropogenic and natural risk factors. The chapter concludes with a discussion on the impact of CWD on cervid population dynamics.

PATTERNS OF EPIDEMIC GROWTH AND APPARENT GEOGRAPHIC SPREAD

The epidemic behavior of CWD resembles that of other infectious diseases of domestic and wild animals, although outbreaks unfold more slowly than seen in some viral and bacterial diseases (Miller et al., 2000). The number of cases tends to increase over time and the geographic area where cases occur also expands where infected host populations are not constrained by fencing. Understanding how localized CWD epidemics grow in prevalence and expand naturally across landscapes can aid in the design and assessment of surveillance strategies and inform the interpretation of spatiotemporal trends at

a larger geographic scale. Knowledge about expected patterns also can be used to assess prospects for natural stabilization and the effectiveness of control efforts (more fully covered in Chapter 6).

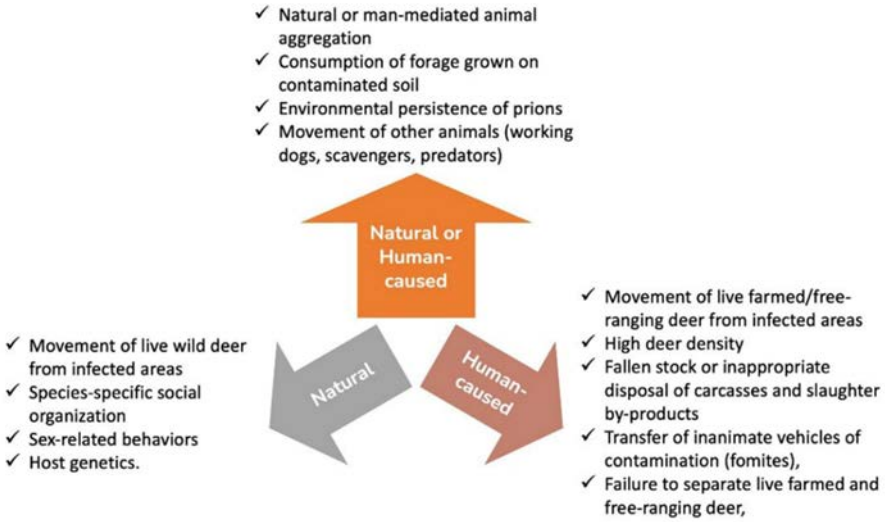


FIGURE 5.1 Summary of natural and human-associated risk factors for CWD spread. Although the figure refers to deer, it is applicable to all susceptible cervids.
SOURCE: Mori et al., 2024.

Epidemic Growth

Epidemic growth has been documented in multiple locations where CWD has become established in captivity or in the wild (see Box 5.1; Miller et al., 2000; Miller et al., 2020; Miller and Williams, 2003; Manjerovic et al., 2014; LaCava et al., 2021; Smolko et al., 2021). As reflected in Box 5.1 and the foregoing references, empirical data from multiple locations show sustained increases over time in either disease incidence or apparent infection prevalence, the proportion of infected individuals in a sample and a proxy for incidence in CWD epidemics (Miller and Wolfe, 2021). In individual captive populations, CWD outbreaks can show relatively steep increases in incidence over several years (Williams et al., 2002; Miller and Williams, 2003; Miller et al., 2006), attributable in part to the smaller and more dense (i.e., concentrated by confinement) host populations but perhaps also to more (or unnatural) opportunities for prion exposure in confined settings (Williams et al., 2002; Miller et al., 2006; Schultze et al., 2023; Mori et al., 2024).

Based on available data, outbreaks among free-ranging herds appear to grow more slowly at the host population/geographic scales upon which surveillance tends to be based. Monitoring of CWD foci in multiple states and provinces over time has yielded data on epidemic growth (e.g., Box 5.1**),** and on natural spatial expansion (e.g., Figure 5.2) where the disease has become established in free-ranging populations (Miller et al., 2000; Manjerovic et al., 2014; Miller et al., 2020; LaCava et al., 2021; Smolko et al., 2021; Thompson et al., 2023). As shown in some of the individual curves from outbreaks observed in free-ranging deer (*Odocoileus* spp.) populations in the figure in Box 5.1, apparent prevalence in uncontrolled CWD epidemics increases slowly and perhaps imperceptibly over the first decade or more, but eventually reach an inflection where growth—approximating exponential—becomes more readily measured from field data. As epidemic growth continues, apparent prevalence can exceed 25 percent (additional examples discussed in later sections). Despite asynchronous starts, CWD outbreaks in free-ranging deer have followed remarkably similar trajectories in multiple locations across the United States.

BOX 5.1
Trends in Apparent Prevalence of Chronic Wasting Disease

CWD shows evidence of slow but sustained epidemic growth over time in multiple locations across the United States. Figure 5-1-1 provides example trends in apparent prevalence of CWD over time in free-ranging deer (*Odocoileus* spp.) populations. The trends demonstrate the similarities observed in CWD epidemic growth across two North American deer species and varied habitats and illustrate the potential duration of CWD epidemics—likely a decade or more in most of the represented locations—at the time the index case is detected.

The data sets presented are from southcentral Wisconsin (CWD detected in 2001; nine monitoring areas; approximately 3,600 square kilometers within four counties spanning approximately 8,700 square kilometers), northwest Colorado (CWD detected in 2002; Williams Fork-Strawberry Creek harvest management units spanning approximately 7,100 square kilometers; Little Snake River-Elkhead Creek spanning approximately 5,700 square kilometers), southwest Pennsylvania (CWD detected in 2012; five counties; approximately 9,400 square kilometers), northwest Arkansas (CWD detected in 2015; five counties; approximately 9,200 square kilometers), and southwest Tennessee (CWD detected in 2018; two counties; approximately 3,600 square kilometers).

Individual points in the graph represent the observed apparent prevalence among harvested adult male deer pooled by area by year, with exponential growth trendlines fitted to the observed data. The solid portion of each trendline covers the time period where field data were available, generally beginning within a year of CWD being detected within each area (i.e., index, or nominal year 0); the broken portions are backward and forward projections of the observed trend assuming the epidemic growth rate to be constant over time. For comparative purposes, the backward projections on trendlines fitted to the six field data sets extend to the point where prevalence approximately 0.001 (1 positive per 1,000 deer present in the area, representing a well-established but nonetheless difficult to detect outbreak in areas of the size of those listed). The epidemic growth trend projected from an early deterministic model of CWD dynamics in northeast Colorado and southeast Wyoming (dashed gray line) is also included for comparison (Miller et al., 2000; EFSA, 2018).

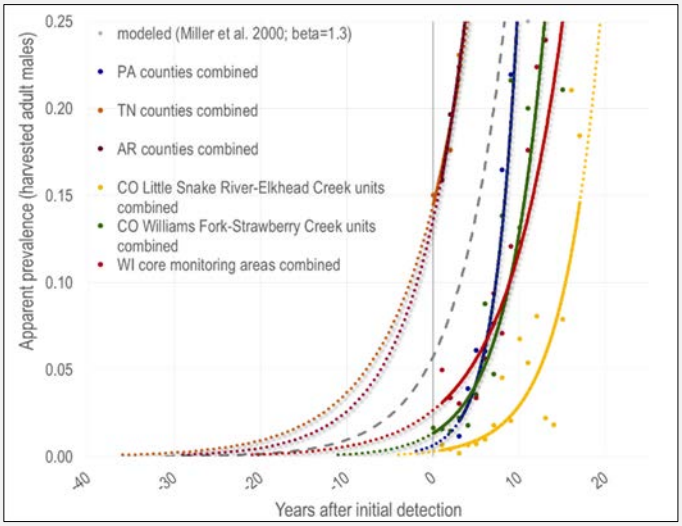


FIGURE 5-1-1 Example trends in apparent prevalence of CWD over time in free-ranging deer (*Odocoileus* spp.) populations in southcentral Wisconsin, northwest Colorado, southwest Pennsylvania, northwest Arkansas, and southwest Tennessee. Points are observed apparent prevalence among harvested adult male deer pooled by area by year, with exponential growth trendlines fitted to the observed data. The epidemic growth trend projected from an early deterministic model of CWD dynamics (dashed gray line; Miller et al., 2000; EFSA, 2018) is included for comparison. SOURCE: Developed by M. W. Miller for this report based on data from the Arkansas Game and Fish Commission[a]; Miller and others (2020), the Pennsylvania Game Commission[b]; the Tennessee Wildlife Resources Agency[c]; and the Wisconsin Department of Natural Resources.[d]

[a] See https://ecommons.cornell.edu/items/c4eea2da-96e6-4184-ae0d-25318e959edc (accessed August 24, 2024).

continued

BOX 5.1 *continued*

[b] See https://pagame.maps.arcgis.com/apps/dashboards/b3c0fd44cc5944ebbc2229ede897b2ae (accessed August 24, 2024).
[c] See https://www.tn.gov/content/tn/twra/hunting/cwd/cwd-in-tennessee.html#distribution (accessed August 24, 2024).
[d] See https://apps.dnr.wi.gov/cwd/summary/county (accessed August 24, 2024).

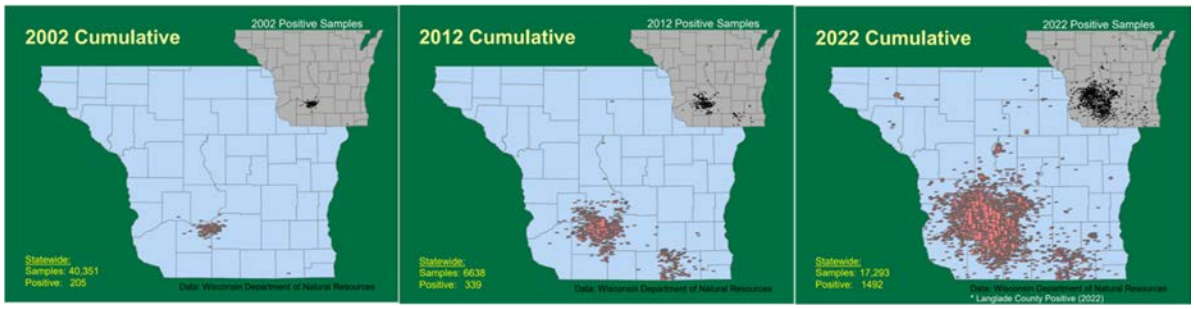


FIGURE 5.2 Observed changes in the geographic distribution of chronic wasting disease (CWD) in the state of Wisconsin during 2002-2022. Field data illustrate spatial expansion of what likely are multiple epicenters over time, the largest of which was first detected in 2001 and was estimated to have started over three decades earlier (Jennelle et al., 2009; Wasserberg et al., 2009; also see Box 5.1).
SOURCE: USGS National Wildlife Health Center, see https://p.widencdn.net/nfe9ku/cwddistribution (accessed May 17, 2024).

Geographic Expansion in Free-Ranging Populations

As the apparent prevalence of CWD increases locally in free-ranging populations, natural movements of infected animals and social overlap with neighboring animal groups leads to geographic expansion of the area affected by CWD (Figure 5.2). With epidemic growth, spatial expansion of an outbreak area tends to occur over several decades (e.g., Jennelle et al., 2009; Wasserberg et al., 2009) and can be accelerated by human activities, as discussed in later sections of this chapter. The rate of natural spread across large geographic areas may be relatively slow: for example, Jennelle and others (2009) estimated an average rate of CWD spread of approximately 0.7 miles (1.13 kilometers) per year in the vicinity of the western core of south-central Wisconsin's largest focus. Seasonal migration or larger host home ranges could drive these rates somewhat higher (Conner and Miller, 2004; Jennelle et al., 2009). Because the state of Wisconsin conducted extensive, systematic statewide surveillance within a few years after first detecting CWD in 2001 (Joly et al., 2009), the observed changes in disease distribution over the ensuing decades are more likely to be real than an artifact of sampling effort. Elsewhere, increasing or expanding surveillance on the perimeter of newly detected foci, a common response to CWD detections (Thompson et al., 2023), may have left the impression that CWD expanded over a shorter period whereas the changing pattern simply reflected the expanded surveillance effort having detected outbreaks in adjacent areas that were already well underway.

Interpreting Geographic Spread at a National Scale

The geographic expansion across 35 states in the United States has occurred over at least six decades, if not longer (Miller et al., 2000; Jennelle et al., 2009; Wasserberg et al., 2009; Miller and Fischer, 2016). Inferring a precise timeline for the arrival of CWD in previously unrecognized areas—for example, based on when CWD was first detection—is problematic (Box 5.1; Miller et al., 2000; Jennelle et al., 2009; Wasserberg et al., 2009; Miller and Fischer, 2016; ESFA, 2018; Thompson et al., 2023). Impediments to accurately describing the timeline of CWD spread in the United States include:

- uncertainty about the epidemiological source(s) of local CWD emergences and incomplete data on movements and distribution of infected animals or other potential infective sources (e.g., scrapie in sheep and goats) over time (see Chapter 2);
- the virtual absence of organized surveillance in either wild or captive cervids prior to the mid-1990s, and inconsistency in surveillance efforts (where practiced) since (Evans et al., 2014; Thompson et al., 2023; Mori et al., 2024; Ruder, Fischer, and Miller, 2024); and
- the practical difficulties in detecting newly established disease foci at the true onset of an outbreak, especially in the free-ranging populations (see Chapter 4).

Limitations in historical and contemporary surveillance, in outbreak investigations, in reporting, and in records on human-assisted translocation all can contribute to uncertainty surrounding precisely when and how CWD has come to occur in a location where it has been detected (e.g., Williams and Young, 1992; Miller et al., 2000; Williams et al., 2002; Joly et al., 2009; Wasserberg et al., 2009; Evans et al., 2014; Miller and Fischer, 2016; Uehlinger et al., 2016; Schultze et al., 2023; Thompson et al., 2023; Mori et al., 2024; Ruder, Fischer, and Miller, 2024). As a result, the precise timing and origin(s) of CWD emergence in the United States and changes in its distribution over time remain uncertain (Williams and Young, 1980; Williams and Young, 1992; Williams et al., 2002; Wasserberg et al., 2009; EFSA, 2017; Miller and Wolfe, 2023). Most non-adjacent free-ranging CWD foci and some outbreaks in captive facilities have no documented times or points of origin or connections to one another (Miller and Fischer, 2016; Schultze et al., 2023; Mori et al., 2024). Although the first recorded encounters and experience with this disease were in Colorado and Wyoming (Williams and Young, 1980; Williams and Young, 1982), available data do not preclude the possibility of CWD originating or emerging independently elsewhere in the United States, either before (Wasserberg et al., 2009) or since, as perhaps happened in Europe (EFSA, 2023).

Published records indicate that CWD-infected and exposed animals were moved via trade or commerce involving U.S. zoos and private collectors by the early 1970s, including at least one international instance (Williams and Young, 1992; Williams and Miller, 2002; Dubé et al., 2006). By the late 1990s, commercial movements of elk had been linked to CWD outbreaks on farms in one Canadian province and five western states (Miller and Williams, 2001; Kahn et al., 2004; Argue et al., 2007). Canadian investigations concluded that CWD likely was imported from the United States in the late 1980s (USAHA, 2001; Kahn et al., 2004; Bollinger et al., 2004), indicating the disease had been spreading undetected in commercial trade for a decade or more. Unpublished records suggest a larger footprint of exposed and infected animal movement was likely. (USAHA, 1998; 2001). Although some outbreaks occurred in commercial facilities with both elk and white-tailed deer (USAHA, 1998), subsequent efforts to investigate and contain CWD in commercial cervid facilities were largely concentrated in the western United States and focused on elk facilities (USAHA, 1998; 2001). The detection of CWD east of the Mississippi River in free-ranging and captive white-tailed deer reported in early 2002 expanded awareness across the United States thereafter (Ruder, Fischer, and Miller, 2024).

In addition to spread of CWD among captive cervid facilities in the late 1990s, undetected CWD likely was expanding in local free-ranging populations where the disease had been introduced (or had independently emerged). However, organized surveillance for CWD in free-ranging populations did not occur outside of Wyoming and Colorado before the mid-1990s and was minimal in most states located east of the Mississippi River before autumn 2002 (Ruder, Fischer, and Miller, 2024). West of the Mississippi River, the majority of CWD surveillance conducted during 1997-2001 was in states with known free-ranging foci or perceived risk due to detection in cervid facilities or proximity to states where CWD was detected in the free-ranging populations (Ruder, Fischer, and Miller, 2024**)**. Multiple newly detected CWD cases and locations during 1997-2002 stimulated marked expansion of surveillance efforts nationwide beginning in autumn 2002 **(**Figure 5.3**;** Ruder, Fischer, and Miller, 2024**)**. Since then, surveillance efforts waxed and waned depending on the availability of federal funds to support sampling and testing, as well as reaction to more new infections (Evans et al., 2014; Thompson et al., 2023).

Chronic wasting disease was documented in one or more commercial cervid facilities in 14 additional states and in the free-ranging populations in one or more locations in 22 additional states during 2003-Spring 2024 (at the time of writing this report). In some cases, the epidemiological connections among infected sites are clear (e.g., USAHA, 1998; Sohn et al., 2002; Argue et al., 2007; Mori et al., 2024). In others, they are not. The greater number of CWD detections since 2002 is sometimes portrayed as evidence of rapid expansion (USGS National Wildlife Health Center, 2024). However, CWD was unlikely to be detected in most states prior to 2002 using the available surveillance data (Figure 5.3; USAHA, 2001; Evans et al., 2014; Ruder, Fischer, and Miller, 2024). In essence, surveillance for CWD began in approximately1997 and surveys had limited detection power in most states prior to 2002, thus the true rate of CWD expansion in the United States cannot be reliably measured. Nonetheless, the available evidence seems sufficient to regard CWD as a widespread animal health problem in the United States that has worsened over multiple decades.

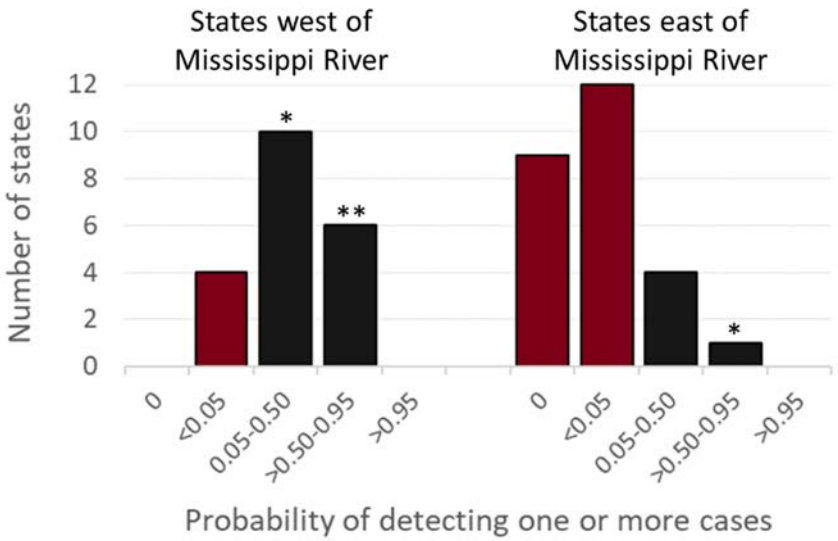


FIGURE 5.3 Inconsistency in probability of detecting CWD in free-ranging cervids at a state-level prevalence of 1 in 1,000 (0.001 or 0.1%) during 1997-2001, summarized from data by Ruder, Fischer, and Miller (2024). Detection probabilities were based on 5-year total number of free-ranging cervid samples screened for CWD in 46 contiguous states where CWD had not been detected in the free-ranging populations, in state reports to the Southeastern Cooperative Wildlife Disease Study. Overall, 25 states had detection probabilities <0.05 (red bars), including nine states that reported testing no samples (detection probability = 0). Asterisks denote the number of states within each bracket that detected one or more CWD cases in the free-ranging populations during the 5-year period. The Mississippi River was used to delineate Western and Eastern states, with Minnesota and Louisiana included in the former. This figure was developed for this report using the data from Table S1 by Ruder, Fischer, and Miller (2024). SOURCE: Data from Table S1 by Ruder, Fischer, and Miller (2024).

DRIVERS OF EPIDEMIC GROWTH AND GEOGRAPHIC SPREAD

The geographic spread of CWD is influenced by both human activities and natural processes. The next sections discuss the anthropogenic and natural risk factors that contribute to the growth and geographic spread of CWD.

Anthropogenic Risk Factors

Humans can move infected cervids—alive or dead—over longer distances and in less predictable ways than cervids are understood to move generally under natural conditions. Inadvertent human transfer

of live, infected cervids is a well-documented mechanism for introducing CWD into distant locations (e.g., Sohn et al., 2002; Argue et al., 2007; Mori et al., 2024) and is a logical explanation of the discontinuous distribution of the disease as observed in North America. Studies indicate that international live animal trade from North America also spread CWD to Asia (Sohn et al., 2002), but there is no evidence that the movement of infected live animals (or carcasses) from North America accounts for its emergence in Europe (EFSA, 2023). Once CWD is introduced into cervid herds, the natural day-to-day and seasonal movements of infected hosts within enclosures or on occupied range leads to local disease transmission and spread (Miller and Williams, 2003; Farnsworth et al., 2006; Jennelle et al., 2009; Xu et al., 2022).

Beyond movements involving infected live cervids, the transfer of carcasses or parts thereof from infected cervids has been suggested as another potential mechanism for spreading CWD in the United States (Gillin and Mawdsley, 2018), but to date the supporting epidemiological data are anecdotal and not definitive (e.g., Kincheloe et al., 2021; Schultze et al., 2023). Similarly, transfer of other tissues, excreta, products, materials, or equipment contaminated with CWD prions have been suggested as potential contributors to geographic spread (Kincheloe et al., 2021; Schultze et al., 2023) but to date evidence of their actual role in spreading the disease is lacking (see Box 5.2).

Given that natural disease transmission factors are more difficult to mitigate than anthropogenic drivers, CWD management policies and regulations are biased toward human-related transmission risks (e.g., live cervid and cervid-part transportation restrictions, bans on urine-based cervid products, baiting and feeding regulations), irrespective of their relative importance to any given disease management scenario. It is likely that the movement of infected but healthy-appearing cervids (either intentional or accidental) has been the most significant of anthropogenic contributors to the introduction of CWD to new locations (Sohn et al., 2002; Kahn et al., 2004; Miller and Wolfe, 2023; Mori et al., 2024), but numerous other factors likely have, and will continue, to accelerate the spread and prevalence of CWD. The role of policy-makers and regulatory agencies in addressing human-caused contributors to disease spread across both free-ranging and captive cervid management jurisdictions will remain critically important even considering the numerous disease transmission modes and potential vectors of CWD that are beyond the influence of existing disease management tools or technologies.

BOX 5.2
Taxidermists, Cervid Meat Processors and CWD

Following the outbreak of bovine spongiform encephalopathy in the United Kingdom, new research and policy emerged to understand and control the risks associated with the downstream processing and use of cattle carcasses and associated materials (e.g., Woodgate and Wilkinson 2021). Although CWD is not currently known to infect humans, there has been little research on current practices of downstream cervid processing operations and how they might contribute to exposure risk among humans or even transmission risk to other cervids. For example, taxidermy has been implicated as having a potential role in the introduction of CWD into captive cervid facilities (Kincheloe et al., 2021). Similarly, cervid meat processors handle large amounts of wild meat intended for human consumption (Hedman et al. 2020), but the meat within these facilities, unless being sold in retail, are considered "custom exempt" and are not under mandatory inspection by USDA (21 U.S. Code § 623). In many processing facilities, cervid meat is processed in bulk (i.e., individual deer often are batched with other deer and a hunter does not necessarily get the meat back from the deer they submitted). Further, processing equipment can become contaminated with CWD prions by meat from CWD-positive cervids and, if not effectively cleaned and disinfected, may subsequently contaminate meat from CWD-negative cervids (Milstein et al. 2024). The committee is unaware of any documentation to date of cases of CWD transmission to humans or cervids originating from these facilities.

Taxidermists and meat processors already play a role in CWD surveillance in some states (Ableman et al. 2019, Thompson et al. 2023), thus there may be opportunities to leverage existing relationships for further research and enhanced CWD control.

Captive Cervid Populations

Key risk factors for CWD transmission among captive cervids primarily are related to direct contact with infected captive or local free-ranging cervid populations (Williams and Young, 1992; Kincheloe et al., 2021; Schultze et al., 2023). Well characterized risks to captive cervids through direct contact include introduction of infected cervids or infected tissues to facilities via live cervid movements or hunting and taxidermy (Williams and Young, 1992; Miller et al., 2004; Kincheloe et al., 2021; Schultze et al., 2023), as well as interactions with infected free-ranging deer through escapes, introductions, and possibly fence line nose-to-nose contact (Kincheloe et al., 2021). CWD prions have been detected in the semen and reproductive tissues of preclinical bucks (Kramm et al., 2019), although any role of transmission through sexual contact or artificial insemination is unknown.

Risk factors associated with indirect transmission are a growing concern, particularly where CWD has been detected in captive facilities with relatively high levels of biosecurity (Kincheloe et al., 2021; Schultze et al., 2023). Indirect contacts, such as through a contaminated food source or perhaps through prion transport by scavengers or other animals accessing captive cervid facilities, are a potential risk (see Figure 5.4) but have not been documented nor verified. Risk factors for indirect CWD transmission can include farm proximity to infected free-ranging deer populations (less than 5 kilometers), presence of other species or scavengers on farm, as well as the presence or location of animal attractants on farm (e.g., location of water source along fence line, forest cover bordering a fence line, or on-premises carcass disposal) (Schultze et al., 2023); however the roles of these potential risks remains unknown. Given the propensity of a variety of materials, including aluminum, rock, cement, polypropylene, stainless steel, wood, to bind and retain prions, common pieces of farm equipment may serve as potential reservoirs for CWD (Pritzkow et al., 2018). Thus, equipment shared between farmed cervid operations may pose a risk for transmission via fomites (i.e., objects or utensils that can carry infectious agents) (Soto et al., 2023d), but this route of transmission has yet to be investigated thoroughly. Recent advances in prion detection via swabs of farm equipment that deer contact offer opportunities to investigate fomite transmission on or between cervid facilities (refer to Chapter 4 for more detail).

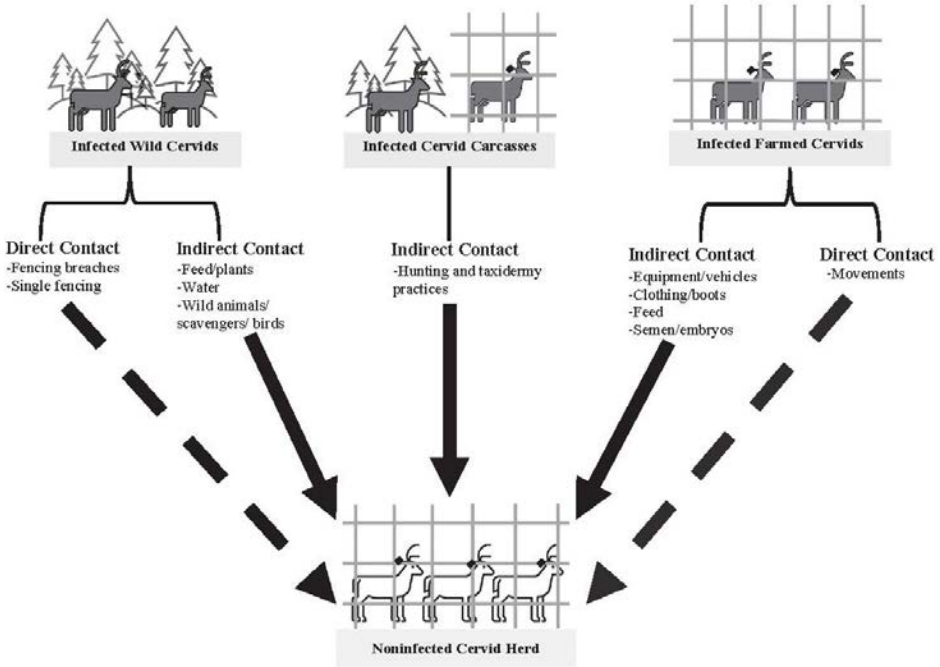


FIGURE 5.4 Potential direct and indirect CWD transmission risks to farmed cervids.
SOURCE: Schultze et al. (2023).

Free-Ranging Cervid Populations

In addition to natural areas where free-ranging cervids gather or visit with higher frequency, human activities that artificially congregate or attract cervids may be risk factors for CWD transmission. These include baiting, supplemental feeding, and mineral supplementation. Supplemental feeding and baiting are well recognized to alter risks of transmitting bacteria, viruses, and prions (see Sorensen et al., 2014 for review; Hines et al., 2007; Thompson et al., 2008) via direct and indirect routes of disease transmission. The risks related to CWD are similar. Environments frequented by deer contribute to CWD epidemics, and sites that congregate deer and elk at high densities are likely accelerating CWD transmission (Miller et al., 2006). Spilled grain associated with agricultural storage bins can be a significant attractant for mule deer (Mejia-Salazar et al., 2018). Supplemental grain feeding through a variety of methods also can artificially increase the concentration and intensity of use by white-tailed deer (Thompson et al., 2008), although no method of artificial feeding was less risky for CWD transmission than natural feeding areas. Supplemental feeding via food plots (rather than grain sources) poses a similar risk for CWD transmission compared to natural deer browsing (Courtney, 2023). Deer using food plots had fewer direct and environmental contacts as compared to those using bait sites. Although more research is warranted to investigate food plots relative to CWD transmission, such findings hold some promise as a low-risk strategy for the nutritional supplementation of free-ranging cervid populations when considered necessary.

The use of cervid urine products as an attractant by hunters has raised concerns among certain individuals within the wildlife disease management community for their as yet undocumented but potential role in CWD transmission. The committee is unaware of any scent or urine facilities that have tested positive under the program to date. As discussed in Chapter 3, infected cervids may shed prions in their urine for months before showing clinical signs of the disease and over the course of infection may shed thousands of infectious doses (Henderson et al., 2015a; Plummer et al., 2017) based on a volume of 10 milliliters of urine contains an approximately 50 percent lethal dose (LD50) for cervidized transgenic mice (Henderson et al., 2015). Because commercial urine lures often come from captive cervids, urine-derived lures could pose a risk for CWD transmission to free-ranging cervids (Miller and Miller, 2016). The relative extent to which prion-contaminated urine contributes to transmission is unclear, but some jurisdictions ban the use of urine and other scent lures to limit potential risk of transferring CWD. Recent hunter surveys demonstrate mixed responses on whether they were willing to abandon the use of urine/scent lures to reduce CWD transmission (Song, McComas, and Schuler, 2019; Seimer, Lauber, and Stedman, 2020). Expanded and standardized regulations around urine sourcing and manufacturing is an alternative approach promoted by the Responsible Hunting Scent Association, which manages the Deer Protection Program (DPP). The DPP is a product certification program that requires cervid herd biosecurity measures above and beyond the APHIS Herd Certification Program, including the screening of urine for CWD prions by RT-QuIC. While this approach might garner greater consumer confidence in product safety, studies have not assessed consumer awareness. There have been no studies to quantify CWD risk reduction through this program.

Carcass Disposal

Miller and others (2004) documented infection of deer maintained in paddocks containing decomposed carcasses of deer that died of CWD. Infection occurred either through direct contact with the carcass remains or exposure to the contaminated environment. The deposition of harvested carcasses via hunting or taxidermy on premises containing captive cervids was associated with CWD detection in a limited number of captive facilities (Kincheloe et al., 2021). The deposition of prions following the decay of CWD-infected carcasses may lead to prion binding and enhanced infectivity in association with certain soil types (Johnson et al., 2007; Kuznetsova et al., 2023). Recent research demonstrates prions associated with carcass tissue decaying on the landscape (Schwabenlander et al., 2024) and even after burial (Soto et al., 2023d), which may serve as local sources of infection based on retention of prions in soil (Jacobson et

al., 2010). Cervids and other ruminants naturally ingest and inhale a significant amount of soil, and the binding of prions to soil particles with subsequent transmission of soil-bound PrP^{CWD} is a likely mechanism of CWD transmission (Beyer et al., 1994; Saunders et al., 2008; Saunders et al., 2012; Smith et al., 2011; see Chapter 3 for more detail). Yet, there is also potential for surface contamination and uptake of prions by plants (Pritzkow et al., 2015) or by translocation and dissemination via scavengers (VerCauteren et al., 2012; Fischer et al., 2013; Nichols et al., 2015). Indeed, the flush of vegetation following carcass decomposition may attract and facilitate transmission to other deer (Towne et al., 2000; Carter et al., 2007). While their role in actual transmission remains unknown, there is active research into prions in soils and plants at known carcass sites.

Anderson (2023) summarizes the risk of CWD transmission via carcass disposal based on the volume of carcasses generated on an annual basis. Hunter-harvested carcasses generated in the 2021 hunting season alone was estimated to be approximately 5.9 million, a figure considered average by the National Deer Association.[1] Even when hunter-harvested carcasses are removed from the local landscape, gut piles are often left by hunters at the harvest site. Although deer have not been observed consuming such remains, direct interactions with residual tissue have been observed and those contacts or consumption of vegetation where tissues decomposed are considered risks for CWD transmission (Miller et al., 2004; Jennelle et al., 2009). Despite the evidence that carcasses or carcass sites are potential risk factors for CWD transmission, the precise mechanisms (e.g., prions associated with soil versus plant material versus residual tissue material), the actual prion load, the long-term risks, and the actual role in natural transmission are unclear. Even so, restrictions on carcass movement and proper carcass disposal have been implemented as tools for controlling CWD in captive and free-ranging herds.

Potential Roles of Feeding practices and Feed Contamination

Multiple presenters and participants of the committee's November 2023 and December 2023 information-gathering sessions (see Appendix B for meeting agendas) suggested that unidentified sources may contribute to CWD outbreaks in some locations. Some form of feed contamination was among the possibilities raised. Despite those voiced suspicions, no epidemiological investigations directly linking CWD outbreaks in captive or free-ranging cervids to contaminated feed were made available to this committee, nor have any been published. However, a case-control analysis based on epidemiological and questionnaire survey data gathered from captive cervid facilities in three states identified the use of "feed harvested from a known CWD-positive area or unknown location" as one of 37 variables potentially associated with CWD-positive herd status among 71 surveyed herds (Schultze et al., 2023, see Appendix A of that document). Committee members also acknowledge additional anecdotal reports or experience along these lines.

The potential role of feeding practices and feed contamination in CWD transmission and geographic spread seem most readily manifested and assessed in captive cervids. Cervids maintained in captivity typically require supplemental feed seasonally or year-round unless enclosures are lightly stocked with animals and sufficiently large and to accommodate year-round natural foraging (Haigh and Hudson, 1993). Farming operations tend to employ more pastoral husbandry systems, whereas shooting/hunting operations tend toward less intensive husbandry within more natural landscapes (e.g., Haigh and Hudson, 1993; Kahn et al., 2004). Naturally occurring or cultivated forage plants (e.g., alfalfa, perennial grasses) also may be available depending on cervid species, location, size, and type of operation (e.g., Brooks and Jayarao, 2008). Some husbandry practices have been suggested anecdotally as potentially increasing CWD transmission risk, but none have been quantitatively demonstrated (Argue et al., 2007; Schultze et al., 2023; Mori et al., 2024).

[1] See https://deerassociation.com/wp-content/uploads/2023/02/NDA-DR2023-FINAL.pdf (accessed July 25, 2024).

Generally, cervid diets in commercial settings include a forage crop (e.g., grass hay or alfalfa, depending on species) and some form of grain or concentrated supplement (Haigh and Hudson, 1993; Kahn et al., 2004; Brooks and Jayarao, 2008; S. Burgeson, email correspondence with the committee, January 2, 2024; S. Shafer, written communication with the committee, January 8, 2024). The extent of animal-origin protein supplement use in commercial cervid operations—currently or historically—is uncertain. Forage crops (e.g., grass hay, alfalfa) are often sourced locally when available in sufficient quantities. More distant sourcing for forage feed may be used or can become necessary when annual weather conditions (e.g., drought, excess precipitation) or climate limit local availability.

Grain and concentrated feed supplement products tend to come from a wider variety of locations (Brooks and Jayarao, 2008). Oats and soybeans are common ingredients in commercial formulations and recommended for supplementation in captive cervids (e.g., Haigh and Hudson, 1993). Some grain and forage crops made available to cervids in the United States are often grown in areas where CWD occurs (see Figure 5.5). Epidemiological investigation of feed and feeding practices are appropriate in the context of their potential contributions to comprehensive control efforts given the potential for overlap between CWD occurrence and production of some crop types, the potential for prion surface contamination and uptake by plants (discussed in Chapter 3), and observations suggesting that feeding and husbandry practices may facilitate prion exposure (Argue et al., 2007; Schultze et al., 2023; Mori et al., 2024).

Natural Risk Factors

In locations where CWD occurs in free-ranging cervid herds, the natural day-to-day and seasonal movements of infected hosts within their occupied range leads to local disease spread across the landscape (Farnsworth et al., 2006; Jennelle et al., 2009; Xu et al., 2022). Interactions within and between neighboring cervid social groups and overlapping home ranges expand the size of affected habitats over time. Exploratory, dispersal, and migratory movements of infected cervids—where they occur—can contribute to somewhat longer-distance spread (e.g., Conner and Miller, 2004; Jennelle et al., 2022), although such distances are still relatively short (e.g., generally tens of miles) in comparison to those achieved with human assistance (e.g., potentially hundreds or thousands of miles; Sohn et al., 2002).

Role of Cervid Population Ecology in Disease Risk

General knowledge about disease transmission indicates that when contact rates increase in association with population density, pathogen transmission is also expected to increase. CWD prion transmission is dependent on direct and indirect interactions among individual cervids (contacts) and thus may increase if contact rates increase. The frequency of infected individuals within a population can influence CWD dynamics (Habib et al., 2011; Storm et al., 2013). However, the density of populations also has an important role in CWD dynamics, and likely a combination of both frequency and density dependent transmission forces drives disease occurrence in deer (Almberg et al., 2011; Storm et al., 2013; Ketz et al., 2019). In areas of relatively low deer density and limited prions in the environment, direct contact among hosts likely has a more significant role than indirect contact with environmental reservoirs (Wasserberg et al., 2009; Almberg et al., 2011; Storm et al., 2013). Therefore, relatively high deer densities increase the probability of contact among deer and thus, CWD transmission if CWD prions are present. In addition, cervid demographics such as sex and age composition may further increase the likelihood of contacts among social groups based on season, reproductive period, and associated behavioral characteristics. These factors are often the target of management actions to effectively reduce direct contact among cervids by reducing overall population density.

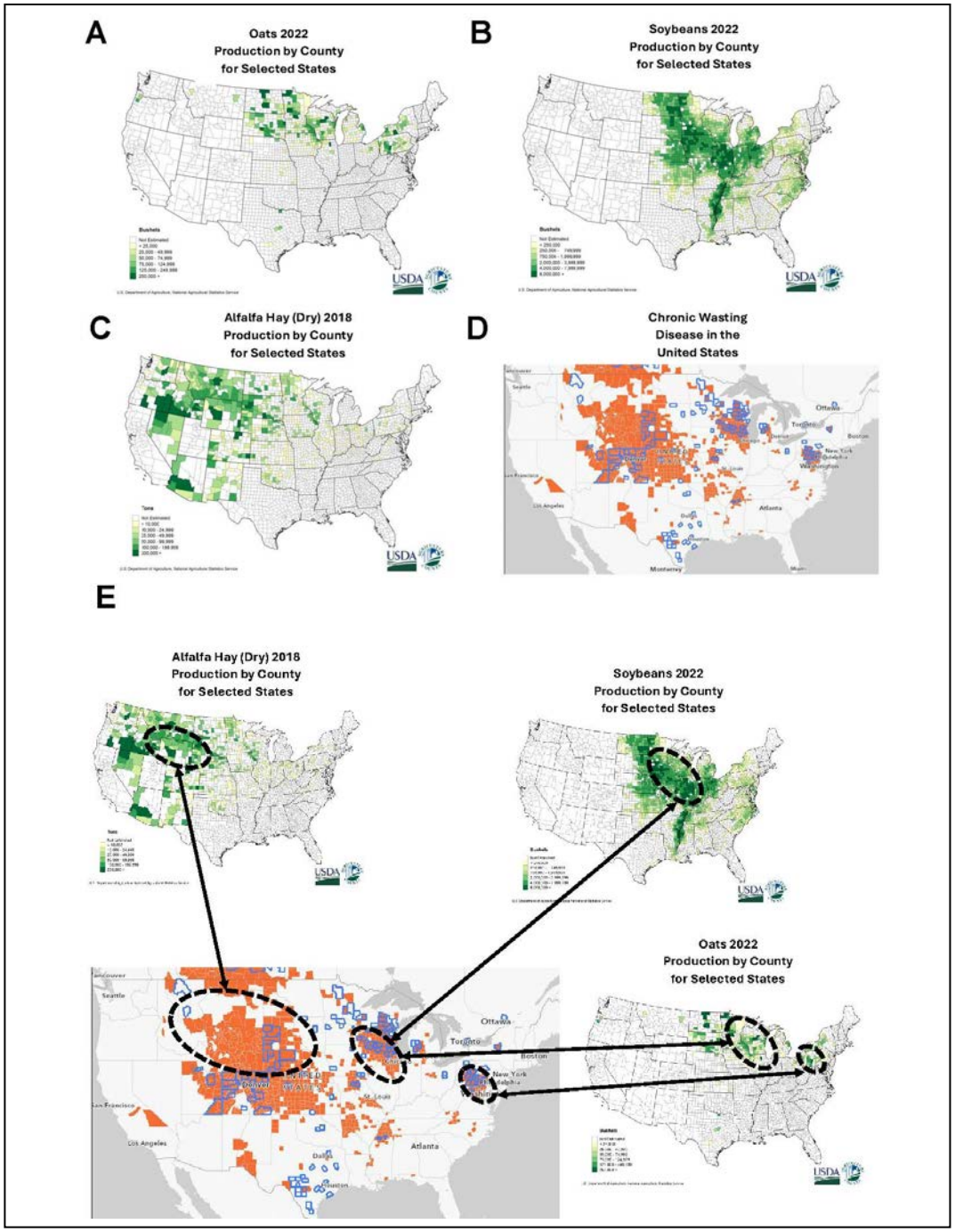


FIGURE 5.5 Relative intensity of production of three of the crops used for cervid feeding, (A) oats; (B) soybean; (C) alfalfa hay within the United States, 2018 or 2022, as reported by the USDA National Agricultural Statistics Service; and (D) the known geographic distribution of chronic wasting disease (CWD) in the United States. Panel E illustrates how some intensive production areas for crops used in cervid feeds may overlap landscapes where CWD occurs.
SOURCES: (A) USDA National Agriculture Statistics Services, see https://www.nass.usda.gov/Charts_and_Maps/Crops_County/ot-pr.php (accessed July 25, 2024); (B) USDA National Agriculture Statistics Services, see https://www.nass.usda.gov/Charts_and_Maps/Crops_County/sb-pr.php (accessed July 25, 2024); (C) USDA National Agriculture Statistics Services, see https://www.nass.usda.gov/Charts_and_Maps/Crops_County/al-pr.php (accessed July 25, 2024); (D) Chronic Wasting Disease Alliance, see https://cwd-info.org/map-chronic-wasting-disease-in-north-america/ (accessed July 25, 2024).

Attempts to quantify transmission risk through evaluating contact rates of free-ranging cervids revealed the dynamic social and environmental components of density-dependent and frequency-dependent disease transmission, which is often scale dependent (Storm et al., 2013) and has been reviewed by various authors such as Ketz and others (2019). Using radio-marked animals and methods such as proximity loggers (contact defined by 3-meter distance between individuals), proximity indices from GPS locations, or specific designations for individual investigations, multiple studies have demonstrated seasonal, demographic, and population density variation in contact rates and CWD risk among cervids.

Dobbin and others (2023) linked locations of contacts to the occurrence of CWD on the landscape, finding that contact probabilities of within- and between-group male paired contacts (dyads) in winter and between-group female dyads in summer were the best predictors of CWD risk. Silbernagel and others (2011) found that close proximity events (CPEs; e.g., contacts) occurred in cropland and wetlands more often than expected given their availability, suggesting a preference among mule deer for these landscapes when available. Kjær and others (2008) found that direct contact rates among white-tailed deer during gestation were highest in forest cover, and then switched to agricultural fields and grasslands during the fawning season. Williams and others (2014) found that daily indirect contact was three times more likely for white-tailed deer than direct contact, but direct contact rates were highest in winter, decreased in spring, bottomed out in summer, and rose in fall. Schauber and others (2007) found contact rates to be 5-22 times greater for deer in the same social group in spring and fall, which then dropped to an even ratio for within group and between groups in summer, suggesting the impact of social grouping on contact rates. The likelihood of contact is associated with various factors that differ widely in individual populations. Habitat preferences, social grouping, and seasonal movements may both explain and constrain relationships and, in conjunction with local host densities and the spatial scale, are crucial for understanding risk of transmission. Interestingly, Janousek and others (2021) found that estimated contact rates were 2.6 times greater for elk at supplemental feeding sites compared to baseline data, indicating an influence of anthropogenic factors on contact rates, similar to increased direct contacts and group size of white-tailed deer at bait sites (Courtney, 2023). While these studies are not exhaustive representations of the range of cervid ecology and dynamics, they represent efforts to quantify disease transmission risk and clarify the mechanisms associated with density or frequency dependent disease transmission. Increased disease risk associated with higher probabilities of contact are closely tied to cervid seasonal resource preferences and breeding cycles that also differ by region. Ultimately, these localized changes in contact rate are presumed to lead to increased transmission of CWD.

Environmental Hotspots

Areas of natural environmental risk for CWD transmission are investigated through two primary lenses: (1) prion shedding and contamination and (2) exposure leading to transmission. Where shedding and contamination are concerned, locations of high cervid density or use have been a focus; whereas environmental characteristics that might contribute to prion persistence and bioavailability (e.g., soil and vegetation characteristics) are being explored relevant to exposure and transmission. Locations of high cervid density or use are considered natural “hotspots”, recognizing the greater potential for CWD deposition into the environment by infected individuals and further transmission to susceptible cervids due to shared use (see Table 5.1). Habitat preferences by cervids and the spatial configuration of those habitats on the landscape can influence the spatial heterogeneity of CWD detections among cervids across the landscape (Evans et al., 2016; Farnsworth et al., 2006).

Observational studies have focused largely on identifying associations between environmental characteristics and CWD prevalence (mostly estimated through harvest-based surveillance). For example, harvest-based CWD surveillance data from Wisconsin and Illinois revealed that larger and compact forested areas, as well as low elevation areas close to large rivers, were associated with higher CWD prevalence in white-tailed deer (O’Hara et al., 2013). In contrast, Evans and others (2015) and Kjær and

TABLE 5.1 Summary of Potential Hotspots for CWD Transmission Among Cervids

Natural cervid hotspots	Study design	Key findings	Citation
Mineral licks	Prion detection	PrP^{SC} in soils and water from mineral licks in CWD foci using PMCA detection.	Plummer et al., 2018
Scrapes	Prion detection	PrP^{SC} by RT-QuIC from soil and tree branch samples at scrape sites	Huang et al., 2024
Bed sites	Behavioral	visited by mule deer, particularly females, with greatest frequency during late gestation and fawning.	Mejía-Salazar et al. 2018
Wintering areas	Spatial/behavioral epidemiology	Winter site fidelity along with limited local movement and interactions, best explained spatial distribution of CWD prevalence in mule deer	Conner and Miller 2004; Farnsworth et al. 2006

Schauber (2022) found forested landscapes had a different relationship with CWD infections, where areas with less forest had a greater risk of CWD detection in deer. In the northeastern United States, the likelihood of CWD infection decreased 6.3 percent for every 1 percent increase of forested land (Evans et al., 2015). Conversely, open and developed habitats increased the odds of CWD infections. Kjær and Schaber (2022) explored landscape configuration further through spatially-explicit simulation modeling and demonstrated that CWD prevalence peaked earlier and at higher levels in areas dominated by agriculture with only forest fragments in comparison to contiguous forest areas. However, these relationships between habitat and CWD risk are likely context specific given the variability in landscapes in which cervids live and CWD has been detected.

CWD surveillance data also has also been used to build empirical evidence supporting the role of soil characteristics in CWD transmission. As mentioned in Chapter 3, prions have been shown experimentally to adsorb strongly to clays or clay soils (Johnson et al., 2006b; Saunders et al., 2009) compared to sandy or other soils, and clay-bound PrP^{Sc} demonstrates higher rates of oral infection (Johnson et al., 2007; Wyckoff et al., 2016). CWD surveillance data for empirical evidence of the hypothesized role of clay in CWD transmission indicated a negative association between soil clay content and CWD in the Midwest (O-Hara et al., 2013). Dorak and others (2017) examined this further in a follow-up study of the persistence of CWD (defined as the detection of greater than three CWD cases in deer in an area, using harvest-based surveillance) in association with soil characteristics in Illinois and found that less than 18 percent clay content and greater than 6.6 pH were the most important soil characteristics associated with CWD persistence. However, the association between clay content and CWD risk differs among studies from different regions. In Colorado, there was a positive association between clay content and CWD in deer (Walter et al., 2011). Other studies suggest soil type or CWD strain characteristics (Saunders et al., 2012) or inherent biases in CWD sampling (Conner et al., 2000; Osnas et al., 2009) among regions may be associated with observed patterns of CWD.

Cervid behavior and movement or habitat use may help identify more local hotspots of potential CWD transmission on the landscape. Camera-trapped female mule deer in a CWD focus in Saskatchewan, Canada visited bed sites during late pregnancy and fawning with greatest frequency (as compared to grain sources, salt licks, browse sites, waterholes, trails, rubs, mortality sites, and bed sites used during any other season) (Mejía-Salazar et al., 2018); although neither the deer nor the visitation sites were tested for CWD. At higher spatial scales, the seasonal movement of mule deer populations in Colorado was examined in association with patterns of CWD. Wintering areas best explained the spatial distribution of CWD in mule deer (Conner and Miller, 2004). Additional research suggested that winter site fidelity, combined with limited movement and local interactions in these areas, may increase CWD transmission within a wintering population (Farnsworth et al., 2006). These authors also suggested that anthropogenic land use may influence natural deer use and density, potentially adding to transmission.

With advances in environmental prion detection technologies, there is a growing body of research that tests hypotheses of prion deposition at predicted cervid "hotspots" such as mineral licks in CWD foci in Wisconsin contained prions (6-19 percent prevalence), based on PMCA detection (Plummer et al. 2018). These may be important sites for indirect transmission given their attraction for cervids and other species and the association of prions with clays at such sites (see above). Deer also create scrapes on the landscape for communication during the breeding season. These sites can be visited and used repeatedly by the same or different individuals during each breeding season (Egan et al., 2023). Preliminary results demonstrate that PrP^{Sc} can be detected by RT-QuIC in soil and tree branch samples at deer scrape sites (Huang et al., 2024), suggesting scrapes might also contribute to indirect transmission of CWD prions via oral or intranasal routes. Much remains unknown regarding natural transmission of CWD among free-ranging populations deer (e.g., direct versus indirect exposure) and specific characteristics of potential CWD hotspots; however, as the capacity to detect CWD in environmental samples continues to expand and be optimized, there may be greater opportunity to explore these questions under natural conditions.

Potential Effects of Predators and Scavengers

In contrast to the clear role of human activities and host animal movements reviewed above, the role of predators and scavengers in spreading (i.e., transporting across geographical regions) or dispersing (e.g., scattering radially around a carcass) CWD prions is undetermined. While there is no evidence of CWD infection in predatory and scavenging mammals or birds (Jennelle et al., 2009; Wolfe et al., 2022), individual animals could disseminate infectious prions either directly by moving carcasses of infected cervids, or by depositing prions in their feces some distance away from the original location of an infected cervid carcass. Any such role in transferring CWD in natural ecosystems, or transmitting CWD to uninfected cervids, would be difficult to document, there is currently no evidence of such exists, and no epidemiological investigations directly linking CWD outbreaks in captive cervids to scavengers or predators have been published. If such transfer does occur, patterns of CWD spread or emergence in new areas may correlate with dispersal or movement patterns of common predators or scavengers. One case-control analysis based on questionnaire survey data gathered from captive cervid facilities in three states identified a possible association between scavenger activity and CWD-positive herd status among 71 surveyed herds (Schultze et al., 2023), although in all, 37 variables were potentially associated with CWD-positive herds.

A variety of mammalian and avian species prey upon and consume free-ranging cervids or consume cervid carcasses on the landscape. These species are essential components of functioning ecosystems and the cervids consumed or removed can be major components of their diet (Newsome et al., 2016; LaBarge et al., 2022). Experimental evidence indicates that some CWD prions remain after passage through the digestive system of coyotes (*Canis latrans*) (Nichols et al., 2015) and cougars (Baune et al., 2021), and that infectious scrapie prions can pass through the digestive system of crows (*Corvus brachyrhynchos*) (Vercauteren et al., 2012). Feces of wild free-ranging coyotes and cougar in areas where CWD occurs in free-ranging cervids have been collected and found to contain CWD prions (Inzalaco et al., 2024).

On the other hand, selective removal of CWD-infected cervids through natural predation has been hypothesized as a mechanism for suppressing the growth of CWD epidemics (e.g., Wild et al. 2011) and could have a part in changing the population stability of elk (Sargeant, Weber, and Roddy, 2011). Limited field data indicate that mountain lion (*Puma concolor*) selectively prey upon CWD-infected mule deer (DeVivo et al., 2017; Fisher et al., 2022; Krumm et al., 2010), lending empirical support to modeled mechanisms for local natural control via predation. Experimental studies also suggest the potential for consumption of prion-laden tissue by predators to reduce prion loads associated with cervid carcasses in the environment (Nichols et al., 2015; Baune et al., 2021). Theoretical models using predator/prey dynamics suggest the role of wolves in removing infected deer from the population could significantly reduce or limit the occurrence of CWD in infected populations (Wild et al., 2011) but, to date, there is no evidence to substantiate this effect in the relatively few areas where CWD and wolves overlap.

Furthermore, the extent to which predators directly or indirectly affect the distribution or availability of prions in the environment is poorly understood. The amount of prion present in cougar feces was considerably less than in the material they ingested, and the relatively rapid passage of food through the gut (1-4 days) limited the time frame when feces containing prions were deposited (e.g., prions were only present in the first defecation after consumption; Baune et al., 2021). Predators and scavengers may dilute prions on the landscape (Fischer et al., 2013) but whether communal rendezvous, denning, or roosting sites could concentrate prions remains unexplored. Other natural phenomena (e.g., flooding, wind, flowing waters) could theoretically contribute to spread but there is no empirical evidence of this.

Potential Risk Associated with CWD Strains

As noted in Chapter 2, CWD strains are epidemiologically relevant for CWD as they can manifest with different virulence, recalcitrance in the environment, host ranges, distribution in animal tissues, shedding potentials, and other biological and biochemical attributes (See Box 5.3 and Otero et al., 2023). Although the current number of strains has not yet been delineated due to the complex nature of strain characterization, there are, minimally, three strains in white-tailed deer (Johnson et al., 2011a, Duque Velasquez et al., 2015; Herbst et al., 2017; Hannaoui et al., 2021, Angers et al., 2010, Bian et al, 2019 and 20xx), at least one strain in mule deer (perhaps similar to the predominant strain in white-tailed deer). There appear to be several strains in elk, likely due to *PRNP* allele differences (O'Rourke et al, 2007, Moore et al., 2018; 2020). As these strains have different biochemical properties (i.e., resistance to proteinase digestion) and differences in host range, the increasing number of strains prevents an understanding of transmission properties, thus limiting the design of strategies on how to control their continued spread.

BOX 5.3
Prion Strains and the Source of their Diversity

The concept of prion strains is an intriguing aspect of prion diseases. Prion strains have long been defined as conformational variants of the misfolded (disease-associated) prion protein (Kascsak, R.J., Rubenstein, R., Carp, R.I, 1991; Bessen and Marsh, 1992; Hill et al., 1997; Safar et al., 2015; Morales, R. 2017). Although this was mostly assumed by indirect, biochemical data, recent reports using high resolution microscopy techniques have demonstrated that different prion strains do, in fact, differ in their conformational motifs (Kraus et al., 2021, Kamali-Jamil et al., 2021; Manka et al., 2023a and b). Prion strains can induce different clinical presentations in the host (Kimberlin et al., 1989; Morales, R. 2017), and may vary in their virulence, persistence in the environment, host ranges, and potential to contaminate other animals and the environment (reviewed in Bartz, 2021). For those reasons, the identification and characterization of prion strains is of capital importance.

The leading source of prion strain variability is variation in the sequence of the prion protein across animal species (Block and Bartz, 2023). Along this line, polymorphic variations in the prion protein within the same animal species also contribute to the diversity of prion strains (Duque Velasquez et al., 2015; Duque Velasquez et al., 2020; Moore et al., 2020; Bian et al., 2021; Angers et al., 2010). Another relevant source of prion strain variation involves inter-species transmissions, where the appearance of diverse prion strains when prions from one animal species successfully infects another one has been reported (Bessen and Marsh, 1992; Duque Velásquez et. a., 2020, Kimberlin et al., 1989; Morales, R. 2017).

All these are particularly relevant for CWD, as i) cervid species differ in their prion protein sequences, ii) several polymorphisms have been identified within individual cervid species' prion proteins, and iii) CWD prions of one cervid species easily infect others. This ability to jump between cervid species may contribute to the emergence and evolution of strains. In other words, CWD presents a unique paradigm in terms of prion strain diversity, as multiple prion strains can naturally emerge. Importantly, just a limited number of prion strains have been identified so far, and their properties and host ranges have not been fully explored.

IMPACT OF CWD ON CERVID POPULATION DYNAMICS

Understanding how animal movement and behavior can be associated with CWD transmission risk and disease effects is important for wildlife conservation and disease management. Ultimately, this information is needed to establish if animals are at risk and if detrimental population effects are possible. The mission for many fish and wildlife agencies is the management and conservation of sustainable healthy fish and wildlife populations for the benefit of current and future publics. Populations of cervids are defined as a single species living in the same place at the same time. These definitions may differ based on the wildlife management goal of interest and they can affect how risks like CWD on a population can be determined. Thus, establishing clear signals of diseases impacts on free-ranging populations are challenging but important for understanding the trade-offs between epidemic growth and control efforts that also may affect cervid abundance.

Robust and reliable estimates of population measures (such as abundance, survival, harvest rates, recruitment (addition of juvenile animals into the breeding population) are critical components for understanding how CWD affects free-ranging cervid populations but are difficult to assess with confidence in free-ranging populations. CWD can result in significant mortality in free-ranging cervids based on studies using radio-marked animals and subsequent postmortem examination. For example, white-tailed deer in Arkansas with high prevalence of CWD in all age classes had a significant increase in mortality of CWD positive deer compared to deer in which CWD was not detected by antemortem testing (J. Ballard, presentation to the committee, December 14, 2023). Reduced survival of prime-aged females and lower recruitment would be expected to result in depressed population growth that could lead to decline. Similarly, overall CWD prevalence in white-tailed deer (n=161) in Wyoming was 35.4 percent, and the two leading causes of mortality were hunter-harvest and CWD (Edmunds et al., 2016). In contrast to the expected pattern of higher apparent prevalence among adult male deer than among females (Miller et al., 2000; Grear et al., 2006; Miller and Wolfe, 2023), this herd showed a notably higher prevalence in females (42 percent) than males (28.2 percent). In total, 17 deer died with clinical CWD (12 females, 5 males). However, bias due to higher harvest of males (76 percent) and hunter preference for adult males may have occurred. CWD-positive deer had a significantly lower survival rate (0.396) than CWD-negative (0.801) and were 4.5 times more likely to die annually. DeVivo and others (2017) found average CWD prevalence was higher in male mule deer (43 percent) than females (18 percent), CWD-positive mule deer were more susceptible to mountain lion predation (n = 20) and harvest (n = 4), and clinical CWD was the second highest cause of mortality (n = 14). CWD-positive mule deer in their study had significantly lower estimated survival rates (0.32) than CWD-negative (0.76) but there was no significant difference (SD) in fawn recruitment rates between CWD positive (0.56, SD = 0.65, 95 percent; confidence interval = 0.30–0.82) and CWD negative mule deer (0.48, SD = 0.65, 95 percent; confidence interval = 0.33–0.63). Similar patterns of disease-associated mortality and increased vulnerability to predation occurred in a nonhunted mule deer herd in Colorado (Miller et al., 2008; Fisher et al., 2022). Finally, researchers evaluated cause of death and pathology for over 1,000 uniquely marked white-tailed deer over a 5-year period in a Wisconsin CWD focus (Gilbertson et al., 2022). They found that infectious disease was one of the leading causes of death and of 245 mortalities with post-mortem CWD tests, 42.4 percent were positive. Prevalence of CWD increased with age among young and prime-aged deer, and some fawns were positive. Similarly, annual survival probabilities (excluding harvest) for a cohort of free-ranging elk declined from 0.97 (Bayesian confidence interval 0.93-0.99) in 2008 to 0.85 (0.75-0.93) in 2010, with declines in survival attributed almost entirely to CWD (Monello et al., 2014). Another striking example of CWD impacts on survival was observed among ranched elk in a Colorado herd, wherein only 16 percent (13 of 82) of CWD-infected elk lived for one year after a positive biopsy, compared to 60 percent of the biopsy-negative elk surviving one or more years in this herd (Haley et al., 2020). Thus, reduced survival in young and otherwise nonfood-limited captive elk further demonstrated the demographic implications of CWD.

Few studies successfully characterized population impacts with sufficient time and disease progression to show how population growth patterns in relatively long-lived species are affected by disease mortality. Funding and political support for such studies typically do not last long enough to determine with confidence changes in population growth rate as a direct result of CWD. Despite these challenges, CWD has been associated with detrimental effects on populations of white-tailed deer, mule deer, and elk in several states. A Wyoming population of white-tailed deer with an estimated average annual CWD prevalence of 23.8 percent declined despite their relatively high reproductive potential (Edmunds et al., 2016). This was evidenced by a reduced annual survival rate, with infected deer being 4.51 times more likely to die and contribute only 0.896 to population growth, which translates to an overall 10.4 percent annual projected population decline (Edmunds et al. 2016). In contrast, annual survival for adult female white-tailed deer in many other regions exceeds 90 percent and population growth rates are above 1.0 even when female deer are actively harvested. A mule deer population in Wyoming, which typically use different habitats and occur at lower densities than white-tailed deer, also declined in the presence of CWD (DeVivo et al., 2017). Finite population growth rate was lower than the previously mentioned white-tailed deer study at 0.79 or a 21 percent annual projected population decline and CWD was a significant contributor through increased mortality of infected animals (DeVivo et al. 2017). Significant decline in a mule deer population in Saskatchewan, Canada with high disease prevalence has been attributed—at least in part—to CWD (Saskatchewan Ministry of Environment, 2020; Stasiak et al., 2023). Similar mule deer declines have been observed across their range for a myriad of reasons, posing an immediate concern for additive mortality due to CWD (Heffelfinger and Krausman, 2023).

Regardless of deer species, the impact of CWD on the life or death of fawns often is negligible (Dulberger et al., 2010; Blanchong et al., 2012; Edmunds et al., 2016; DeVivo et al., 2017), indicating that the adult mortality accompanying increased prevalence, in concert with other demographic and environmental factors, is likely driving effects on the population growth rate (Ketz et al., 2019). In the absence of hunting losses, Rocky Mountain elk had slightly more resilience to CWD at the population scale, with population growth rates near 1.0 (population stability). However, clear negative effects on population parameters would be more likely as prevalence increases in combination with food and resource limitations or with the addition of hunting (e.g., Monello et al., 2014; Sargeant et al., 2011). Ketz and others (2019; see Figure 5.6) projected population growth trajectories with increasing prevalence based on point estimates of prevalence and population growth reported for cervid populations in Colorado and Wyoming where CWD has existed for several decades. These examples provide a stark one-way projection for other populations that may reach similar prevalence levels.

Simulation-based population models also have projected negative population impacts (see reviews by Ketz et al., 2019; Winter and Escobar, 2020; Belsare et al., 2020; Thompson et al., 2024; Kjær and Schauber, 2022). The modeled consequences underscore the potentially significant ramifications of CWD and its risk to wild cervid populations and the systems (both ecological and socioeconomic) that rely on them. Further, efforts to use limited empirical cervid population data in combination with advanced quantitative models have addressed some crucial gaps in existing knowledge. For example, resource use and selection by cervids likely interacts with prevalence and demographics on the landscape to affect differences in population impacts (e.g., Evans et al., 2015; Kjær and Schauber, 2015). The management and regulated harvest of cervid populations is then another factor that influences the trajectory of a cervid population, as demonstrated via modeling (e.g., Foley and others 2016; Gross and Miller 2001). For example, the models described by Foley and others (2016) illustrated that a deer population without CWD or harvest was projected to increase 1.42 percent annually within 25 years, whereas modeled populations with CWD and without harvest showed markedly lower annual growth (0.41, -1.72, or –10.33 percent, where negative values reflect a population decline) at low, medium, or high CWD prevalence, respectively. Conner and others (2021) and Potapov and others (2016) also highlighted the role of harvest management to affect CWD prevalence. Increasing the number of hunters and the harvest total of male deer led to lower prevalence the next year. Harvesting animals closer to peak breeding times lowered the CWD prevalence, which aligns well with most agency regulated hunting

periods. These studies collectively suggest that CWD prevalence and its impact on deer populations are influenced by a multitude of factors, and comprehensive and long-term approaches to measuring impacts and subsequent management actions are critical.

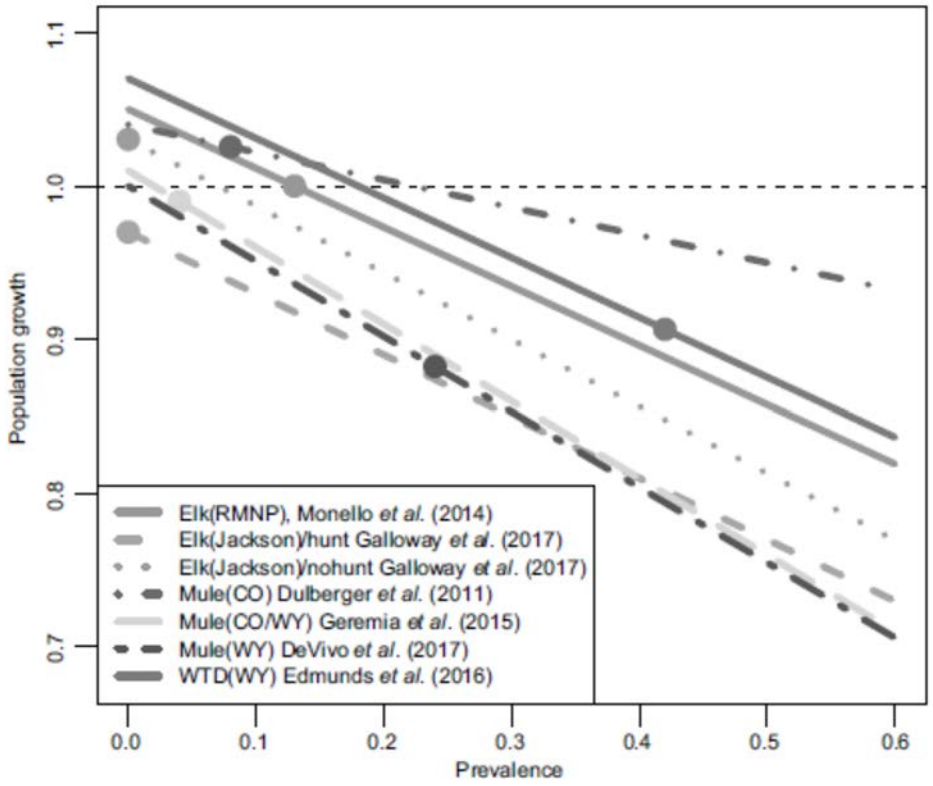


FIGURE 5.6 Graph showing modeled population growth rate trends (i.e., linear projections) with increasing prevalence of CWD. Population growth rates tend to decrease with increasing prevalence of CWD in multiple simulation models for different species, populations, and CWD prevalence. Points on lines in the graph indicate estimated CWD prevalence at the times population growth rates were estimated.
SOURCE: Ketz, Storm, and Samuel (2019).

6
Effectiveness of Interventions to Control or Reduce the Transmission and Spread of CWD in Captive and Free-Ranging Cervids

Chapter Highlights

- Despite knowledge gaps, strategies based on well-established principles for animal disease management are available to help prevent, control, or limit chronic wasting disease (CWD) transmission and spread. Their long-term effectiveness continues to be evaluated.
- The USDA Animal and Plant Health Inspection Service (APHIS) supports the voluntary, state-administered CWD Herd Certification Program (HCP) with the goal of providing "a consistent, national approach to control the incidence of CWD in farmed cervids and prevent the interstate spread of CWD."[1] Fewer than half of the captive cervid herds in the 28 participating states are enrolled in the HCP. CWD has been detected in some HCP-certified herds (<1 percent annually during 2018–2023).
- The CWD HCP is based on logical disease-management principles and likely has been beneficial in helping control CWD in the US captive cervid industry, but the lack of accessible data makes its effectiveness difficult to quantify.
- CWD cases among HCP herds suggest that biosecurity measures may need to be reconsidered and that indirect transmission within and among herds may need to be better understood.
- CWD control among free-ranging cervid populations is more difficult given the geographic extent and severity of some outbreaks, as well as the diversity of landscapes, cervid species, and the varying interests and support of affected parties. Tailored and standardized adaptive CWD/cervid management strategies among jurisdictions and situations have substantial advantages and merit.
- Measuring the effects of CWD-control strategies for free-ranging herds is challenging due to underestimation of CWD occurrence (scope and scale), constraints on the implementation of control programs, and a lack of sustained support among management agencies and interested and affected individuals and groups.
- Research to broaden possible control options (e.g., vaccines, therapeutics, genomics, and environmental decontamination) may be beneficial, but development times will likely be lengthy and field applications—especially in free-ranging populations—may present further challenges.

As described earlier in this report, CWD was recognized as an animal health problem in affected captive cervid facilities even before its etiology and infectious nature were appreciated (Williams and Young, 1980; Williams and Young, 1992). Attempts to contain and control CWD in captive cervids herds date back to the 1980s, with limited effectiveness (Williams and Young, 1992; Miller et al., 1998; Dubé et al., 2006). Underestimating prion contamination and persistence in the environment and the unavailability of antemortem diagnostic tools were arguably the two main impediments. Since the late 1990s, the recognition that CWD was more widely distributed in captive and free-ranging cervids has compelled broader and more organized investigation, surveillance, control, and containment efforts (reviewed by Williams et al., 2002; Uehlinger et al., 2016; Thompson et al., 2023; Chronic Wasting Disease Task Force, 2002). The nature of the CWD prion and insidiousness of resulting clinical disease,

[1] See https://www.aphis.usda.gov/livestock-poultry-disease/cervid/chronic-wasting/herd-certification (accessed October 24, 2024).

its slow epidemic dynamics, its presence in both free-ranging and captive host populations and, importantly, its ability to contaminate and persist in the environment for extended periods, present formidable challenges to containing and controlling CWD, and to assessing the efficacy of such efforts.

This chapter briefly overviews principles of disease management in domestic and wild animals, summarizes the accessible knowledge about approaches for controlling other transmissible spongiform encephalopathies (TSEs), the control of CWD, and the apparent effects of control interventions in captive and free-ranging settings, and identifies gaps and limitations in knowledge about CWD control and how those might be remedied moving forward. The committee stresses its reliance on "accessible" knowledge for this summary, knowing there is additional research and data that could inform more broadly if it were made available (e.g., through public databases or reports or via publication in the scientific literature).

GENERAL PRINCIPLES OF DISEASE MANAGEMENT IN DOMESTIC AND WILD ANIMALS

Despite the challenges associated with managing CWD, the well-established "rationale, strategies, and concepts of animal disease control" (e.g., Martin, Meek, and Willeberg, 1987) generally apply. Although CWD has some unique characteristics regarding transmission and spread, it generally adheres to the same epidemiological principles as other infectious diseases. The traditional epidemiologic triad model holds that infectious diseases result from the interactions among an agent, a host, and the environment. Transmission occurs when the agent (prion) leaves the host/reservoir (CWD-infected cervids) through a portal of exit and is conveyed by some mode of transmission (direct or indirect) and enters through a portal of entry (e.g., oral) to infect a susceptible host. This sequence is referred to as the chain of infection. The components of the chain regarding CWD have been described in previous chapters. All known infectious diseases—regardless of whether the agent is a virus, a bacterium, a fungus, a parasite, or a prion, or whether the disease involves humans or animals—can be described and explained by this foundational principle (Dicker, 1992).

It follows that knowledge about the portals of exit and entry and the mode of transmission provide a basis for determining appropriate control measures (e.g., Martin, Meek, and Willeberg, 1987; Dicker 1992; Wobeser, 2007). In general, control measures are usually directed against the segment of the infection chain that is most susceptible to intervention. Efforts to manage diseases in domestic or wild animals are motivated by anthropocentric concerns about the consequences–absent management–for humans and/or animals they care about (Martin, Meek, and Willeberg et al, 1987; Wobeser, 2007). Several considerations thus underlie animal disease management (Martin, Meek, and Willeberg, 1987; Wobeser, 2007; Stephen, 2022). As summarized by Wobeser (2007) in the context of attempts to manage a wildlife disease, these considerations include: desirability, feasibility, beneficiaries, costs and benefits, availability and viability of approaches or tools, objectives and extent, and measures of "success."

The objectives for disease management fall under three broad concepts (e.g., Martin, Meek, and Willeberg , 1987; Wobeser, 2007): prevention (i.e., invoking measures to exclude or prevent the introduction of the disease agent of concern), control (i.e., invoking measures to reduce the frequency of an existing disease to levels that are biologically or economically justifiable or tolerable), and eradication (i.e., invoking measures to eliminate the disease agent of concern from a defined geographic area, which may be local, national, or global). Of these, eradication requires the most extreme measures in applications to either domestic or wild animal populations (Martin, Meek, and Willeberg, 1987; Wobeser, 2007). In the context of CWD, it seems noteworthy to consider that the features of potentially eradicable infectious diseases— per Yekutiel's (1980) critique on the subject—include having detrimental effects sufficient to justify the economic impacts, relative ease of case detection and surveillance, and the availability of at least one effective tool for breaking transmission. The pursuit of a disease eradication campaign also requires careful consideration of: the rationale for its preference over control, whether administrative, operational, and fiscal resources are adequate to support the entire undertaking, and the potential for direct or indirect adverse effects of such efforts (Yekutiel, 1980). As observed by Wobeser (2007, p.195), choosing among the three basic objectives for disease management "depends upon many

factors including the presence or absence of the disease in the area, the length of time the disease has been present, the frequency of occurrence and distribution of the disease, the species affected, the availability of suitable methods for detection, diagnosis and management, the desirability or need for management, and the ability to convince others of this need. Often an overall program may involve aspects of prevention, control and eradication, with different techniques being used at various stages of the program."

As detailed in later sections of this chapter, the approaches that have been applied in attempts to manage CWD under one or more of the foregoing objectives are common to efforts directed at managing other animal diseases. Specific disease management activities (adapted from Martin, Meeks, and Willeberg, 1987) include:

- lethal removals via means including slaughter (as well as culling and hunting), applied selectively or nonselectively at scales ranging from individuals to populations (i.e., "depopulation"),
- animal movement restrictions (sometimes termed "quarantine") for infected/exposed individuals, populations, or regions,
- reduction of contact via physical barriers or other biosecurity measures,
- "chemical" applications (e.g., disinfectants, therapeutics),
- modifications of host resistance (e.g., via vaccines, genetic selection),
- environmental manipulations,
- biological control, and
- education (of humans).

These approaches have been (and are) used in various combinations in attempts to manage animal diseases (see examples and additional references in Martin, Meeks, and Willeberg, 1987; Haigh and Hudson, 1993; Wobeser, 2007; Gillin, 2022). Regardless of the approaches adopted, additional key features of attempts to manage disease in either domestic or wild animals include having sufficient time to affect change in the dynamics of the targeted disease agent, a means of assessing responses to intervention (e.g., changes in disease frequency or distribution over time), and the ability (and willingness) to modify or adapt approaches to evolve with changing circumstances (Martin, Meeks, and Willeberg , 1987; Wobeser, 2007; Gillin, 2022; Stephen, 2022).

Several approaches for reducing transmission and spread of CWD have been identified and are reviewed in this chapter. These include restricting the movement of infected, potentially exposed, or untested cervids and cervid carcasses, and reducing or eliminating infected cervid herds and exposure opportunities (i.e., limiting the local abundance, sex-age composition, aggregation, and captive holding of cervids in places where CWD has become established) to help suppress transmission and further geographic spread. Management is underpinned by comprehensive surveillance and monitoring to reliably detect CWD cases and foci in captivity or in the wild during the early stages of an outbreak and to assess responses to control. Although the knowledge and tools to accomplish each of these have been available for some time, their implementation has thus far been limited because they are not practical on a sufficiently large scale, are not palatable to local (or broader) constituents who have a say in adopting policies and enacting management, or are not sustainable because support for them wanes over time (e.g., Holsman et al., 2010; Miller and Fischer, 2016; Uehlinger et al., 2016; Smolko et al., 2021; Thompson et al., 2023). The actions envisioned as necessary and feasible from afar (e.g., from outside a jurisdiction or a local community) often do not enjoy the same level of acceptance in the places where such actions are to be implemented. Experience to date suggests top-down approaches for controlling CWD are less likely to be sustained or to succeed than approaches that have been developed and accepted more locally (Western Association of Fish and Wildlife Agencies, 2017).

CONTROL OF OTHER TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

It is tempting to look to how other transmissible spongiform encephalopathies (TSEs), such as classical scrapie and classical bovine spongiform encephalopathy (BSE) were controlled for guidance in the control of CWD, although differences in the epidemiology of each of these TSEs makes extrapolation difficult (see Table 2.1). Control programs for scrapie and BSE have seen many successes. The incidence of classical scrapie has been substantially reduced in the United States because of culling infected flocks and promoting selective breeding of resistant phenotypes. Classical BSE was controlled among cattle in the United Kingdom and elsewhere in Europe through changes in animal feeding practices (Wilesmith et al., 1988; Wilesmith et al., 2000). However, scrapie control has been largely successful in the United States because of the design of a nationwide eradication program where all adult sheep and goats bought, sold or otherwise moved between flocks and farms are officially identified for implementation of disease control strategies, including a nationwide slaughter surveillance program (USDA National Scrapie Surveillance Plan, 2022). Appendix E of this report provides details about the scrapie eradication program. The national program for CWD focuses on control, not eradication, and enrollment, participation, and surveillance are not consistently mandated (more detail provided in this chapter). Forward strides in scrapie control were also made because susceptibility among sheep and goats have strong genetic links (Goldman et al., 1994; Hunter, 1997; Baylis and Goldman, 2004; Nodelijk et al., 2011). Although variations in the cervid PrP genotype confer differences in CWD progression, complete genetically based resistance to infection (and ongoing transmission) has not been observed in cervids.

BSE was controlled because the cause of transmission was identified (i.e., feed containing contaminated animal tissue and bone meal) and effectively eliminated (Wilesmith et al., 1988; Wilesmith et al., 2000). Whole-herd culling was also conducted but, because there is no evidence of horizontal transmission of BSE between cattle, that intervention may not have been necessary if infected animals could be identified and removed from the human and animal food chains (Detwiler et al., 2000). In contrast, CWD transmission among cervids is not limited to the consumption of animal protein, but rather involves avenues of both direct (animal to animal) and indirect (environment to animal) transmission (more detail in Chapter 3); thus, CWD control necessitates a multimodal control strategy. BSE is zoonotic (unlike scrapie) which also dictates the control response. Finally, unlike scrapie and BSE, which are TSEs confined to captive livestock, the transmission of CWD among free-ranging cervid populations adds additional complexity. See Appendix E for more information about BSE control.

GAUGING THE EFFECTIVENESS OF CWD INTERVENTIONS

Drawing conclusions about the state of knowledge regarding CWD and the "effectiveness" of interventions to reduce transmission and spread of CWD—as the study committee is directed in its statement of task (see Box 1.2)—is not straightforward, particularly if effectiveness is considered at a national scale. As discussed in previous chapters, prion transmission cannot be measured directly in natural exposure settings. Moreover, the continuous and intensive monitoring that would be necessary to detect new cases over time (incidence) is not practicable on a broad scale in free-ranging settings or in some captive settings. Because CWD incidence strongly correlates with apparent prevalence (the proportion of a sample of animals that is infected; Miller and Wolfe, 2021), observed prevalence trends have been used to monitor epidemic dynamics in free-ranging cervids. Surveillance and monitoring are therefore integral to understanding the effects of management strategies for CWD control in the wild, as is true for assessing management of most wildlife diseases (Wobeser, 2007). The main laboratory tests used for CWD detection (e.g., immunohistochemistry [IHC] and enzyme-linked immunosorbent assay [ELISA]) are considered sufficiently reliable, have been available for over two decades, and were instrumental in controlling other animal TSEs (see Chapter 4 for descriptions, Appendix D for a summary of published diagnostic platforms, and Appendix E for further discussion of scrapie and BSE control programs). Similarly, the principles of CWD surveillance and monitoring are well-established (see Chapter 4), although how surveillance and monitoring are conducted has varied over time and across

jurisdictions (see Chapters 4 and 5). Reliably comparing CWD prevalence data over time and across jurisdictions requires consistency in data gathering methods, thus presenting potential challenges to assessing the effect of interventions—or lack thereof—on epidemic trends. Additionally, accurate measurement of CWD's geographic spread requires reasonable certainty about its spatial distribution over time (see Chapters 4 and 5). Measuring CWD's population and economic effects and responses to interventions present even more complex challenges (see Chapter 7). Because jurisdictions may design their surveillance and monitoring programs to meet different objectives, particular care may be required in comparing data derived from different jurisdictions when trying to understand CWD patterns and management responses at a national level.

Beyond the foregoing challenges, no common standards of acceptable response thresholds for CWD prevalence or related disease outcome metrics have been established by animal health and management authorities. Each sets its own disease management targets and approaches, as well as surveillance and monitoring schedules. This is not entirely surprising given that each management jurisdiction (i.e., fish and wildlife management or agricultural agency) operates under unique sets of circumstances based on CWD's known distribution, prevalence, affected species, cervid population and movement dynamics, as well as the political climate, legal constraints, and cultural expectations within a jurisdiction (Holsman et al., 2010; Miller and Wolfe, 2023; Thompson et al., 2023). Consequently, even where effects are measurable, the perceived effectiveness of control efforts directed toward CWD will tend to be situational and will likely be influenced by whether or not interested parties share common management goals.

As will be illustrated in the rest of this chapter, it is not only the management techniques for curbing CWD that merit further assessment and development but also the realistic goals for its control at small and large spatial scales. Effectiveness is relative and has been assessed in that context for this report.

APPROACHES FOR CONTROLLING CWD IN CAPTIVE CERVIDS

Organized investigation, surveillance, control, and containment efforts for CWD in captive cervids have been in place since the late 1990s, first in Canada and subsequently in the United States (USAHA, 1998; USAHA, 2000; Chronic Wasting Disease Task Force, 2002; National CWD Plan Implementation Committee, 2002; Kahn et al., 2004). Possibly the most important component of CWD management is prevention, which is dependent on measures found across livestock farming and health management including reliable means of both herd and animal identification, current records of animal inventory and movement, appropriate biosecurity, and accurate disease surveillance. With the discovery of CWD on a property, these measures also afford a better understanding of disease epidemiology. Once the disease is discovered, management relies on herd-level quarantine, animal trace-outs, and in many cases depopulation, with further work done to characterize the extent of the outbreak and minimize its impacts on both captive and surrounding free-ranging cervid herds. Indemnity is provided where available to both compensate the herd owner and encourage disease reporting and subsequent control measures.

State-Federal Program to Address CWD Among Captive Cervid Populations

In 2003, the USDA Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) began development of what is now the CWD Herd Certification Program (HCP[2]; USDA-APHIS, 2019). This is a cooperative program involving the APHIS, state animal health and wildlife agencies, and captive cervid owners. The goal is to provide uniform methods to minimize the incidence and spread of the disease. The program is administered by states, with federal oversight to assure appropriate biosecurity, accurate disease surveillance, and host genetics in application of the program throughout the states. Participation in the program is voluntary for both the state and producer, however interstate

[2] See:www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/cervid/cervids-cwd/cervids-voluntary-hcp for links related to the program (accessed May 21, 2024).

movement is restricted to certified herds. The legal requirements for the HCP and interstate movement requirements are outlined in Title 9 of the Code of Federal Regulations parts 55 and 81. The HCP Standards outline the minimum requirements to certify captive cervids for interstate movements. At this time, no tribal agencies or captive cervid facilities owned by tribes were known to be participating in this program; although it appears that there is nothing that would preclude their participation. Inclusion of tribal agencies or cervid facilities may necessitate some revision to state-federal program standards where states have no jurisdiction on tribal lands.

The HCP for captive cervids applies well-established principles for animal disease control (e.g., Martin et al., 1987; Wobeser, 2007) and mirrors other USDA-listed notifiable diseases, including bovine brucellosis,[3] bovine tuberculosis (USDA, 2005), and highly pathogenic avian influenza (USDA, 2020) in several respects. The HCP's core elements are common to programs enacted in Canada, the states of South Dakota and Colorado, and elsewhere beginning in the late 1990s (USAHA, 2000, 2001; Kahn et al., 2004; Argue et al., 2007). The aforementioned prevention, detection, investigation, and response practices, enhanced by the federal HCP enacted in 2012, have generally aided in the early discovery and rapid containment of the disease on cervid farms across the United States. For herds not enrolled in the HCP, states may impose specific regulations that aim to limit the risk of herd-to-herd disease transmission within the state and quickly contain any cases identified. Some captive herds, for example those part of the Deer Protection Program, go beyond the requirements of the HCP to avoid prion contamination of their products (see Box 6.1 and Chapter 5).

BOX 6.1
CWD Management in the Scent Industry

The white-tailed deer scent urine industry, specifically the Responsible Hunting Scent Association and those associated with the Deer Protection Program, takes several steps to minimize or eliminate the risk of CWD prion contamination in their products. In addition to the routine postmortem testing of deer that is standard practice with deer farming operations, deer farms producing urine-based scents are commonly "closed herds"—that is they neither take in new cervids from outside the herd nor sell cervids to other facilities. This reduces the risk of CWD being imported or exported. The facilities and cervids are more frequently inspected and evaluated for general health than standard deer farming operations, with biosecurity practices typically greater than those suggested by the USDA HCP. Importantly, the urine products themselves are tested using the highly sensitive RT-QuIC assay to verify the absence of amplifiable CWD prions[4] (S. Burgeson, presentation to the committee, November 16, 2023).

Although not all cervid farms in all states in the United States are required to participate, the HCP has provided a foundation for CWD management practices for captive cervids at a national scale. The program provides requirements for herd enrollment in the program, including varying aspects of animal and herd level identification and inventories, biosecurity, and surveillance. Herd inventories and animal movement records, which must be consistently maintained to remain in the program, provide important data that assist trace-outs investigations, where necessary. Interstate movement of captive cervids requires the herd has been certified in the HCP and is regulated by the USDA. The HCP provides some guidance on biosecurity, including recommendations for barriers to reduce contact with outside wildlife, in most cases a single row of fencing at least 8 feet in height. Additional biosecurity measures, for example, a second row of fencing, farm-specific clothing, limitations on individuals with access to the farm, feed sourcing, and isolation areas for cervids showing signs of disease, are not currently discussed in the HCP and are not consistent practices across the cervid farming industry. Finally, the HCP requires consistent

[3] See https://www.aphis.usda.gov/livestock-poultry-disease/cattle/bovine-brucellosis (accessed August 6, 2024).
[4] See https://www.cwdfacts.org/rt-quic-urine-testing/ (accessed August 26, 2024).

postmortem testing of all cervids, 12 months or older, that die. Accommodations may be made for missed tests on deceased (or escaped) cervids through "replacement" postmortem testing from comparable cervids in the herd, or a reduction or loss of herd certification status. Postmortem samples, collected by certified personnel, are submitted to one of the 32 approved NAHLN laboratories[5] across the country, with any positive cervids ultimately confirmed by the NVSL.

With the discovery of CWD within any captive herd, HCP-enrolled or otherwise, the herd is initially placed under state-authorized movement restrictions to limit the further spread of disease while investigations into animal movement on ("trace-ins") and off ("trace-outs") the farm are performed to identify source and destinations herds of CWD-positive and potentially exposed cervids.[6] Trace-in/trace-out facilities are likewise placed under quarantine, with similar restrictions on animal movement. Further investigations that focus on potential routes of CWD introduction may be performed. All facilities placed under quarantine have options that may include: (1) complete depopulation and postmortem testing, (2) a 5-year continuance of the quarantine under the guidance of a mutually agreed upon herd plan, or (3) a live-animal testing protocol which considers the range of prion genotypes on the premises and the time since reported positive cases or exposure.[7] Trace-in/trace-out facilities which hold potentially exposed cervids are given the option of killing and testing those cervids. Live animal testing in conjunction with genetic analyses may be performed in some cases in white-tailed deer herds. Quarantined herds may be removed from quarantine if no additional positive cases are identified over a five-year period, during which time their herd status is listed as "suspended." Depopulated herds are provided with federal indemnity, when available, with costs of testing and disposal covered by the USDA. Indemnity is provided on a case-by-case basis, with preference given to herds enrolled in the HCP, among other factors.

Following depopulation, further guidance on proper disposal of carcasses, site decontamination and continued biosecurity is provided under the HCP. Carcass disposal methods are determined prior to depopulation and include incineration, alkaline hydrolysis treatment, or disposal on-site or in an approved landfill. Each method has corresponding strengths and weaknesses with regards to cost and prion inactivation effectiveness. Site decontamination considers farm equipment and infrastructure (e.g., tractors, fencing, barns) as well as animal pastures, and may include approaches like chlorine treatment of exposed equipment and surfaces as well as soil removal or re-tilling. Finally, facilities under quarantine are required to maintain perimeter fencing and otherwise minimize the risk of further exposure to free-ranging cervids outside of the fence. Restocking with CWD-susceptible species is highly discouraged following depopulations, with further cases likely even after a period of perhaps 10 or more years based on data available for classical scrapie (Georgsson et al., 2006; Hawkins et al., 2015).

Metrics for evaluating the effectiveness of programs like the HCP typically include considerations of the number of farms enrolling (or reenrolling) in the program each year, the number of positive cases or herds identified each year (for both enrolled/certified and non-enrolled herds), a cost-benefit analysis of the program and its potential impact on case reduction, and satisfaction polling of invested parties. Assessments of disease prevention programs consider data both prior to program initiation and throughout its deployment.[8] Data on many aspects of the overall effectiveness of the HCP are either limited or were unavailable to the present study committee. As of December 2023, there were 28 states participating in the HCP. Although the total number of herds nationwide at that time is unknown, over 1,600 herds were enrolled and 85% of these herds were certified (personal communication, T. Nichols, June 20, 2024). The yearly frequency of CWD-positive HCP certified herds has remained relatively constant at <1 percent since 2018 (personal communication, T. Nichols, March

[5] See https://www.aphis.usda.gov/labs/nahln/approved-labs/cwd (accessed May 22, 2024).
[6] A tutorial on animal disease traceability has been developed by the USDA and is available here: https://www.aphis.usda.gov/sites/default/files/slowburn/story_html5.html (accessed August 26, 2024).
[7] Detailed in the USDA Chronic Wasting Disease Program Standards. See https://www.aphis.usda.gov/sites/default/files/cwd-program-standards.pdf (p.43; accessed October 24, 2024).
[8] See https://www.ruralhealthinfo.org/toolkits/health-promotion/5/measures-for-evaluating (accessed May 22, 2024).

12, 2024) (see Table 6.1). Also of note is the steady decline in the number of herds certified through the HCP over the past five years (Table 6.1), which may indicate some level of dissatisfaction with the program (S. Schafer, personal communication, December 14, 2023) or other factors. In addition to reporting instances of CWD among HCP certified herds, the program works continuously with states to monitor and improve compliance.

TABLE 6.1 Approximate Numbers of CWD-positive Captive Cervid Herds Identified in the United States[a]

	Number of CWD Positive Herds			
Federal Fiscal Year	**Not enrolled in HCP**	**HCP enrolled but not yet certified**	**HCP certified (% of total HCP certified herds)**	**Total HCP certified herds**
2018	7	0	6 (0.3%)	1,875
2019	9	1	9 (0.5%)	1,748
2020	17	2	7 (0.4%)	1,723
2021	23	3	8 (0.5%)	1,520
2022	12	2	6 (0.4%)	1,537
2023	15	3	7 (0.5%)	1,420

[a] Based on data from https://www.aphis.usda.gov/sites/default/files/status-of-captive-herds.pdf (accessed May 22, 2024) and T. Nichols, personal communication (June 20, 2024). Data on the total number of captive herds by state across the United States were not available.

Other metrics that could provide insights into the effectiveness of the HCP, where such data are available, include the number of captive herds in states that are not participating in the HCP, the timeliness of case identification and herd quarantine or depopulation, when applied, CWD prevalence at time of depopulation, as well as the number of farms traced out from a positive herd and their eventual outcomes. Estimates on the time between disease introduction and identification of a positive case (e.g., through correlation between genetic background and disease stage) and between initial detection and quarantine or depopulation could allow regulatory agencies to assess improvements in these metrics over time. Similarly, herd prevalence estimates at time of depopulation, after controlling for time since first detection, would offer further insights. A continuous reduction in the number of herds traced out and placed under quarantine is an important indicator of curbing the expansion of CWD, and the rapid identification of affected cervids—through live animal testing within herds and prior to animal movement—could be an important verification tool.

Despite the implementation of the national HCP, newly detected CWD infected herds continue to be discovered (Kincheloe et al., 2021; Schultze et al., 2023). Traditionally, efforts at CWD prevention have focused mainly on precluding direct contact between non-infected captive cervids and infected captive or free ranging cervids or carcasses thereof. More recently, the potential role of indirect contact with infectious materials originating from, but in the absence of, infected cervids is becoming more apparent, including avenues of indirect contact with infected free-ranging cervids (Schultze et al., 2023) or with other infected cervid facilities (C. Seabury, presentation to the committee, December 14, 2023). Examples of indirect contact that may present a potential risk include exposure to contaminated objects such as feedstuffs (including forage), water, non-susceptible wild or domestic animals acting as mechanical vectors, equipment (e.g., farm, handling, veterinary), vehicles, clothing, and potentially semen and fetal material.

The effectiveness of state-federal programs is dependent on timely detection of CWD infections, however early detection in a captive herd may be limited because:

- USDA-approved diagnostic tests are implemented primarily postmortem and can only be performed by USDA-approved laboratories.

- Captive cervids may be exposed to other sources of CWD agent in some locations given the local prevalence and distribution of the disease among free-ranging cervids**.**
- Antemortem cervid tissues need to be submitted for testing in a timely manner by captive cervid owners to preserve the diagnostic quality of the specimens.
- Extremes in ambient temperatures and the size and terrain of cervid enclosures affect if, how, and when dead cervids may be found and specimens collected.

State-federal programs also are limited by gaps in the current understanding of the epidemiology of CWD. The source of exposure in newly infected herds is often undetermined. The long incubation period of the disease, extended periods during which infected animals shed CWD prions, the variable expression of the CWD based on individual animal genetics, and the persistence of the prion in the environment all complicate the ability for captive-cervid owners participating in the program to comply vigilantly with program requirements.

State Captive Cervid Programs

The success of the CWD HCP depends on cooperation between state agencies and USDA. However, intrastate movement of captive cervids does not fall under the control of the HCP, and movements of captive cervids within a state may be frequent and over long distances (Makau et al 2020). The committee determined, through expert elicitation (see Appendix B for meeting agendas) and web resources (e.g., of the Michigan Department of Natural Resources[9]) that state-based programs range from the complete banning or elimination of captive cervid farming to nonparticipation in the HCP. Several western states have CWD programs for captive cervid facilities that predate the federal HCP, and in some cases have more stringent requirements (e.g., mandatory participation, herd certification required for movements within the state; Michigan Department of Natural Resources, 2021[10]). The present study committee was unable to acquire epidemiological data or summaries of captive-cervid facility CWD data collected through either the HCP or state-based programs to assess the influence of those programs on infection patterns. Nor could the committee mount a full review of state programs and regulations or determine if comparable state data were available to evaluate program efficacy in CWD control in those programs. A recent review of CWD in captive cervid herds suggests that such data are lacking (Mori et al., 2024).

Alternative Management Efforts

Alternative management approaches for captive facilities with CWD-positive cases and outside of HCP guidance protocols have not been described or assessed in detail. The approaches have typically involved either (a) live animal testing and removal of positive cases (i.e., 'test-and-cull') or (b) genetic selection approaches to reduce the herd-level susceptibility to CWD. In an example of a test-and-cull approach, ranched elk were screened yearly by RAMALT biopsy and a subset of CWD-positive animals were euthanized (Haley et al., 2018; Haley et al., 2020). This was largely unsuccessful, and the herd was ultimately destroyed after the 3-year study. Studies relying on artificial selection, discussed in additional detail in a later section of this chapter, have focused on the *PRNP* gene alone or in tandem with genome-wide markers and are in the early stages of investigation (Haley et al., 2021a; Seabury et al., 2022). Future studies may consider combining these two approaches to help improve disease management. Early efforts to control CWD in captive research herds have been summarized (Williams and Young, 1992; Miller et al., 1998), and updates on those efforts could be useful in understanding long-term prospects for

[9] See CWD and Cervidae regulations in North America at https://www.michigan.gov/dnr/managing-resources/wildlife/wildlife-disease/disease-monitoring/cwd/cwd-hunting-regulations/cwd-and-cervidae-regulations-in-north-america (accessed August 27, 2024).

[10] See https://www.michigan.gov/dnr/managing-resources/wildlife/wildlife-disease/disease-monitoring/cwd/cwd-hunting-regulations/cwd-and-cervidae-regulations-in-north-america (accessed August 6, 2024).

control of CWD in captive herds. It seems plausible to the committee that reports or summaries of other nonfederal herd management plans and practices to limit case occurrence in captive facilities also may be on file in some states. Compilation of unpublished data as well as summary and evaluation of practices to control CWD could benefit national control efforts by providing assessments of intervention effects.

APPROACHES FOR CONTROLLING CWD IN FREE-RANGING CERVID POPULATIONS

With few exceptions, CWD has spread geographically and increased in prevalence over time—albeit to varying degrees—following the detection of a new focus, irrespective of disease control measures implemented by wildlife management authorities (Uehlinger et al., 2016). Multiple reviews of CWD management strategies for free-ranging cervid populations have observed that the tools for controlling CWD are limited, and some that seem promising have generally been too inconsistently applied across time, geographical area, and affected species to reliably determine their ultimate effects in curbing transmission and geographic spread (Miller and Fischer, 2016; Uehlinger et al., 2016; Fischer and Dunfee, 2022; Thompson et al., 2023). Additionally, wildlife managers consistently acknowledge that most of their efforts to control CWD in past decades have been constrained and confounded by a host of challenges that include, but are not limited to, compromised project design and implementation, underestimation of the extent of the disease-affected area or cervid populations and duration of the disease prior to its detection, inadequate duration of application, flawed assumptions of disease emergence drivers, political and socioeconomic conflicts, and regulatory agency jurisdictional limitations (Miller and Fischer, 2016; Uehlinger et al., 2016; Thompson et al., 2023).

The foregoing variables in CWD management implementation have complicated the ability of wildlife managers and decision makers to objectively determine the effects of CWD management strategies through time and across jurisdictions. Changes in the approaches, intervention frameworks, scientific and cultural knowledge, and political influence also have occurred since the discovery of CWD in North America (Williams and Young, 1992; Williams et al., 2002; Miller and Fischer, 2016; Western Association of Fish and Wildlife Agencies, 2017; Gillin and Mawdsley, 2018; Thompson et al., 2023). The current recommended strategies, techniques, and procedures are based on established principles for animal disease control (e.g., Martin et al., 1987; Wobeser, 2007; also see Box 6.4 and the section covering GENERAL PRINCIPLES OF DISEASE MANAGEMENT... earlier in this chapter) and incorporate CWD-specific scientific and practical knowledge, but do require further application and assessment to fully understand their ultimate utility in long-term CWD management (Western Association of Fish and Wildlife Agencies, 2017; Gillin and Mawdsley, 2018; Fischer and Dunfee, 2022).

As noted previously, no common standard of acceptable response thresholds for CWD prevalence or related disease outcome metrics have been established by management authorities. Each wildlife management agency or authority sets its own disease management targets and sampling schedule. This is not surprising given that each U.S. management jurisdiction (i.e., state or federal wildlife management or agricultural agency, or tribal organization) operates under a unique set of circumstances based on the known distribution of CWD, prevalence, affected species, cervid population and movement dynamics, political climate, and cultural expectations within that jurisdiction (Holsman et al., 2010; Thompson et al., 2023). Consequently, an adaptive approach to CWD management efforts based on sound epidemiological principles (e.g., Martin et al., 1987; Wobeser, 2007) is likely to be effective for wildlife management agencies faced with a fluctuating and complex matrix of biological and social factors that emerge and persist following the discovery of CWD within their jurisdiction (Western Association of Fish and Wildlife Agencies, 2017; see Box 6.2 for a brief description of adaptive management). That said, a number of general management strategies have apparent effects on CWD prevalence and rate of spread (Table 6.2; Miller and Fischer, 2016; Fischer and Dunfee, 2022). These have been reported in formal and informal assessments and, along with other relevant disease management practices (e.g., Martin et al., 1987), have served as a foundation for best practices in CWD management technical guidance (Chronic Wasting Disease Task Force, 2002; National CWD Plan Implementation Committee, 2002; Western Association of Fish and Wildlife Agencies, 2017; Gillin and Mawdsley 2018; Fischer and Dunfee, 2022).

BOX 6.2
Adaptive Management

Natural resource management necessarily involves decision making in the face of the uncertainties that are part of complex evolving ecosystems. Complicating those decisions are resource constraints, different jurisdictional priorities, and the desire to minimize negative social and economic impacts. Resource management decisions need to be made in a manner that is consistent with an understanding of the system being managed and a range of policy goals, and, in the case of disease management, need to be based on sound epidemiological principles. Although there are many definitions of adaptive wildlife management, it can be described as a structured iterative plan based on defined goals (e.g., Stankey et al., 2005) that is iteratively and responsively modified based on observations (i.e., learning from experience). As knowledge is gained through management and observation, uncertainties are reduced, and decisions can be refined. Holling (1978) described adaptive management as an interactive process that integrates the concepts of adaptive assessment and policy design "focused through policy concerns.". For wildlife management and research in particular, numerous examples have been described and continue to be used (Williams and Brown 2013).

The National Academies of Sciences, Engineering, and Medicine (2004) described the characteristics of a well-structured adaptive management process based on the adaptive management literature of the day which are still applicable today. Such processes are those for which (a) management objectives are regularly reviewed and revised; (b) a baseline model of the managed system has been developed from which decision effectiveness can be measured; (c) a range of possible management decisions has been evaluated for consistency with goals and in consideration of the model; (d) metrics for evaluating outcomes are established and appropriate monitoring data are collected from the outset; (e) mechanisms for modifying decisions are established and incorporated into planning; and (f) a participatory structure is established for shared communication and learning with interested and affected parties.

Adaptive management techniques are not unique to wildlife management and have been applied to management decisions in an array of disciplines. Examples of adaptive management approaches applied to CWD modeling include those by Wasserberg and others (2009) and Galloway and others (2021). Over time, the effectiveness of such approaches in the management of CWD needs to be quantitatively examined and documented as part of the ongoing nature of adaptive management. Various CWD management strategies have been suggested (Western Association of Fish and Wildlife Agencies, 2017).

Following from the concepts outlined by Thompson and others (2023), the measures implemented by wildlife management agencies to control CWD can be grouped into three broad categories covering those that are (1) preventive and preceding detection, (2) responses following initial detection, or (3) adaptive responses for sustained control. This categorization provides a useful temporal framework with which to review and assess CWD management actions for free-ranging cervids undertaken by wildlife management agencies. The next sections summarize some of these approaches.

Preparing for Rapid Response

Agencies have taken multiple actions intended to prevent or detect CWD introduction and lay a foundation for eventual responses to detection within their jurisdictions. These include regulatory interventions to reduce the potential of introducing CWD to new areas (e.g., live cervid and cervid carcass part movement restrictions, bans on baiting and feeding free-ranging cervid, cervid part disposal restrictions, and mandatory submission of hunter harvested cervids for CWD surveillance programs), weighted risk-based surveillance strategies to detect early disease occurrences or outbreaks, and development and publication of a CWD response plan. Box 6.3 is only one example of a collaborative effort by bnations to, in this case, conduct surveillance, although other concerted efforts of enhanced CWD surveillance have been conducted by tribal agencies across the U.S. with federal support (Chronic Wasting Disease Task Force 2002; USDA APHIS, 2021; Schwabenlander et al. 2022).

TABLE 6.2 Apparent Effects of Strategies Implemented to Manage Chronic Wasting Disease (CWD) in Free-ranging Cervids, including Measured Effect Sizes (where reported)[a]

Management tool	CWD status	Reported apparent effect(s)	Host species	Jurisdiction	References
Spatially-targeted culling +/- hunting	recent exposure or introduction from identified source	Removals in the immediate vicinity of a known exposure source and few or no free-ranging cases appeared to prevent local outbreaks from becoming established based on subsequent monitoring.	white-tailed deer	New York Minnesota Québec	Hildebrand et al., 2013; Evans et al., 2014; New York State Department of Environmental Conservation, 2018; Minnesota Department of Natural Resources, 2019; Gagnier et al., 2020; also see state reports in Fischer & Dunfee, 2022
Spatially-targeted culling	Emerging	Across 6 core management units, the odds of removing a CWD-positive deer via a spatially targeted herd-reduction program was ~3x higher than via hunting (odds ratio 3.37; 95% CI: 1.96-5.79).	mule deer white-tailed deer	Alberta	Smolko et al., 2021
Hunting	emerging to well-established	Across 32 management units where starting prevalence was ≤5% among male deer, increasing hunter and harvest numbers led to lower prevalence in the following year (range of ß values -0.043 to -0.218; $P \leq 0.023$); harvesting male deer later in the year also led to lower prevalence in the following year, with a smaller but additive estimated effect.	mule deer	Alberta, Colorado, Nebraska, Utah, Wyoming	Conner et al., 2021
Hunting	emerging to well-established	In six of 12 hunt areas, the average number of licenses inversely correlated with prevalence among deer harvested 1-2 yr later; changes in license numbers positively correlated with the chance of adult male deer harvested 1-2 yr later being free from apparent infection (i.e., test-negative), with odds ratios ranging from 1.2 (95% CI: 1.17-1.24) to 17.04 (CI: 5.00-58.10).	mule deer	Colorado	Miller et al., 2020

continued

TABLE 6.2 *continued*

Management tool	CWD status	Reported apparent effect(s)	Host species	Jurisdiction	References
Spatially-targeted culling	well-established	When both states were actively culling (2003–2007), there were no statistical differences between state CWD prevalence estimates. Wisconsin government culling concluded in 2007 and average prevalence over the next 5 years was 3.09 ± 1.13% with an average annual increase of 0.63%. During that same 5-year period, Illinois continued government culling and there was no change in prevalence.	white-tailed deer	Illinois Wisconsin	Manjerovic et al., 2014 (also see **Figure 2** from Thompson et al., 2023)
Spatially-targeted culling + hunting	well-established	Management aimed to reduce CWD transmission between 2000 and 2005 via a combination of focal culling and a broader increase in female harvest, decreasing overall deer abundance by about 25%. Analyses carried out shortly thereafter suggested reductions in deer density had made little impact on CWD prevalence. However, subsequent assessments suggest those management actions may indeed have attenuated the outbreak. Surveillance on four winter ranges conducted during 1997–2002 indicated overall CWD prevalence in female deer >1 year old was 0.08 (95% credible interval 0.06−0.11), whereas prevalence during 2010–11 was lower (0.04, 0.02−0.07). An independent comparison showed a comparable decline in prevalence among harvested male deer in the same area (2002: 0.15, 95% binomial confidence interval 0.11-0.20; 2017: 0.06, 0.04-0.09).	mule deer	Colorado	Conner et al., 2007; Geremia et al., 2015; Miller et al., 2020

Selective removals via 'test & cull'	well-established	Over a 5 yr period, 48-68% of the estimated number of adult (≥1 yr old) deer were screened annually via tonsil biopsy immunohistochemistry (IHC), collecting 1,251 samples from >700 individuals and removing IHC-positives. Among males, prevalence during the last 3 yr of selective culling was lower (one-sided Fisher's exact test P~0.01) than in the period prior. Prevalence among females during the before and after periods were equivalent (P=0.77). Relatively higher annual testing of males (mean 77%) compared to females (mean 51%) may have contributed to differences in responses to management.	mule deer	Colorado	Wolfe et al., 2018

[a] Although the examples do not represent true field experiments, the observations illustrate the potential for some measurable level of CWD control to be achieved and offer a basis for designing management experiments that would allow for more rigorous assessments and comparisons of the apparent effects of various CWD management techniques.

BOX 6.3
The Midwest Tribal CWD Surveillance Network

There is growing interest in the surveillance and management of CWD among tribal nations as CWD encroaches on more Native lands where tribal members hunt.[a] Schwabenlander and others (2022) surveyed midwestern tribal natural resource managers in 2020 on their efforts and priorities related to CWD outreach, surveillance, and management. They found that despite limited agency capacity and funding, these CWD-related activities were greatly desired. At that time, a newly formed Midwest Tribal CWD Network had formed among eight tribal nations in Minnesota, one in Michigan, two intertribal agencies, and the University of Minnesota (UMN). This network has since expanded and includes an additional tribe each in Minnesota and Michigan. The network is a collaborative effort of tribal managers and CWD experts to conduct surveillance through resource sharing that overcomes individual agency capacity limitations and incentivizes tribal hunter participation (Moore, 2023). This program was launched with funding from the United State Fish and Wildlife Service Tribal Wildlife Grants program and supplemented through the USDA's cooperative agreements program. To date, no CWD detections have been made on tribal lands through this surveillance network (T. Wolf, personal communication, May 11, 2024; Yoder, 2023), but the need for CWD management plans for tribal lands managed by these agencies is recognized (Schwabenlander et al., 2022). This need has generated new research efforts by members of the tribal-university partnership to draft community-informed plans that may serve as a blueprint for how individual tribes might adopt prevention and control strategies for CWD that integrate cultural values and priorities (Faust et al., 2023; Faust et al., 2024).

The success of these endeavors through the Midwest Tribal CWD Network (as evidenced by annual increases in sampling and tribal partners) has been based on the co-development of a program that prioritizes individual and collective tribal needs related to CWD (accomplished through ongoing and transparent communications and mutual respect) and leverages the individual strengths of its partners (T. Wolf, personal communication, January 5, 2024). However, CWD is considered an environmental justice issue for Native American tribes through environmental abrogation of subsistence rights, and current CWD funding strategies are not considered sustainable for ongoing, meaningful CWD surveillance and management (Moore, 2023; Schrage, 2023). The USDA-APHIS cooperative agreements program, which is responsible for most of the current CWD funding available to tribal and state agencies (Chiavacci, 2022), is distributed through a competitive program with a complex and time-consuming application process and reporting requirements. The Midwest Tribal CWD Surveillance Network is one example of efforts to overcome barriers to Tribes, but additional engagement of Tribes by federal and state agencies to better understand the CWD-related goals and needs of tribes across the United States may still be warranted.

[a] See https://www.usgs.gov/media/images/distribution-cwd-relation-tribal-lands-us (accessed October 26, 2024).

Sites heavily contaminated with CWD-infected cervid feces, saliva, or decomposing carcasses can become environmental reservoirs for infectious prions (Miller et al., 2004; Plummer et al., 2018) and serve as sources of indirect transmission of infections prions (Miller et al., 2004). Locations where free-ranging cervids are artificially congregated by anthropogenic lures (e.g., food, mineral licks) thus may exacerbate direct and indirect transmission in areas affected by CWD. For these reasons, bans or restrictions on free-ranging cervid baiting and feeding and on high-risk cervid carcass and on the movement and disposal of carcass and carcass parts are generally considered prudent practices to reduce the risk of CWD transmission and introduction, although their effects remain incompletely understood. Rigorous surveillance strategies implemented prior to CWD detection (e.g., Samuel et al., 2003; EFSA Panel on Biological Hazards, 2023) have facilitated the apparent eradication of possible CWD outbreaks, at least at local levels (New York State Department of Environmental Conservation;[11] Fischer and Dunfee, 2022; Thompson et al., 2023). To date, the only examples of CWD management strategies affecting the apparent local eradication of CWD (New York, Minnesota) have reportedly occurred

[11] See https://dec.ny.gov/nature/animals-fish-plants/wildlife-health/animal-diseases/chronic-wasting-disease (accessed August 12, 2024).

because of rapid management response following the very early detection of a disease outbreak in the wild (Thompson et al., 2023). Over half of the initial detections of CWD in free-ranging cervids in the United States have occurred via state fish and wildlife agencies testing hunter-harvested cervids (Thompson et al., 2023), although this may reflect the wide reliance on this surveillance approach rather than its higher sensitivity (e.g., EFSA Panel on Biological Hazards, 2023).

Fischer and Dunfee (2022) and Gillin and Mawdsley (2018) noted that the establishment of a CWD response plan prior to its detection has proven important in multiple states. Individual states have reported that doing so provided a foundation for establishing rapid and more widely accepted disease management actions and regulations that yielded greater likelihood to mitigate future disease spread and engender more support for long-term strategies (10-20 years) needed to minimize CWD's impact on affected populations (Fischer and Dunfee, 2022). The critical importance of a publicly and politically supported CWD response plan cannot be overstated given that the values, attitudes, risk acceptance, and perception of loss vary greatly within and among hunters, landowners, wildlife enthusiasts, and other vested segments of the public (Schroeder et al., 2021; Meeks et al., 2022). The rapidity, duration, and resource commitment required to effectively address and manage a CWD occurrence in a free-ranging cervid population are greatly impacted by the public and political support for agency-led CWD management actions (Miller and Fischer, 2016; Thompson et al., 2023). Thus, the creation and publication of a CWD response plan prior to the discovery of CWD in a state allows time to implement methodical and genuine public engagement, education, input, and coalition building needed to gain support for strategies regarded as most likely to effectively manage CWD upon its detection (Gillin and Mawdsley, 2018; Fischer and Dunfee, 2022; Thompson et al., 2023). See Chapter 7 for discussion on decision analytical processes for identifying and addressing conflicting priorities in decision making.

Responses Following Initial Detection

Response actions following the initial detection of CWD in free-ranging cervids within a management jurisdiction (tribal lands or U.S. states) have included increased risk-based sampling to delineate disease-affected areas (e.g., mandatory submission of hunter harvested cervids), initiation of CWD control measures in those areas (e.g., modifications to hunter harvest rates, culling to reduce population density or to target localized disease foci), initiation or expansion of CWD-related regulations (e.g., live cervid and cervid carcass part transportation restrictions, cervid baiting and feeding bans, cervid hunter regulations), and communication and engagement with the public and interested and affected parties. Following first discovery, wildlife management agencies may implement strategies to simultaneously increase sampling of cervids and reduce affected population density within a designated radius of the initial detection by increasing hunter harvest limits or targeted culling (Thompson et al., 2023). The enhanced, targeted and ongoing sampling and removal of cervids in affected and surrounding areas allows agencies to adapt or modify management strategies in response to additional data on or changes in the patterns of CWD distribution or occurrence or where hunting is not an option.

Current best practices (Western Association of Fish and Wildlife Agencies, 2017; Gillin and Mawdsley, 2018) advocate for the delineation of a CWD management zone or initial response area under the authority of the state wildlife management agency where a multi-year surveillance effort can be employed to reliably document disease prevalence and distribution with relative precision. This often requires a significant increase in testable samples acquired through hunter-harvested or agency-culled cervids. Establishing initial estimates of CWD prevalence and distribution is considered critical to formulating appropriate disease response measures as well as establishing baselines that can be used to reliably assess future impacts of new management efforts and strategies (Western Association of Fish and Wildlife Agencies, 2017). In conjunction with this, CWD management best practices (e.g., Gillin and Mawdsley 2018) recommend reducing the probability of contact through reduction of local cervid population densities. Uehlinger and others (2016) noted that direct cervid population management (intensive culling, in particular) is the most empirically supported CWD control strategy documented to date. For example, a "test-and-cull" approach analogous to that applied in captive settings was feasible

and effective in CWD suppression in males (among which management was more intensive) with limited application to a free-ranging mule deer herd (Wolfe et al., 2004; Wolfe et al., 2018), although in practice this approach seems best suited to small geographic areas or as an adjunct to other broad-scale control measures like hunting. An approach for achieving such reduction at a larger geographic scale has been to combine targeted removal (i.e., culling) in high-risk or high prevalence locations and an increase in regulated hunter harvest within a generous border around the primary area of concern (e.g., see state reports in Fischer and Dunfee, 2022). Some field studies have documented that targeted removal can maintain low disease prevalence when implemented consistently over time and focused on the highest-risk demographics (Manjerovic et al., 2014; Thompson et al., 2023, Figure 6.1). However, these efforts must be maintained at adequate levels as some states have seen increases in apparent prevalence when targeted removal was abandoned (Thompson et al., 2023**,** Figure 6.1). As these efforts have not always been consistently applied, or sufficient time has not been afforded to assess outcomes (e.g., see Conner et al., 2007 vs. Geremia et al., 2015), the reported results have been mixed (e.g., Smolko et al., 2021; Thompson et al., 2023). Sustained hunting pressure also has been shown to suppress epidemic growth in affected mule deer populations when adequately applied (Miller et al., 2020; Conner et al., 2021).

Once CWD has been established in a free-ranging cervid population, wildlife management agencies typically impose regulations aimed at reducing the risk of indirect disease transmission caused by human actions (Fischer and Dunfee, 2022; Thompson et al., 2023). Plummer and others (2018) demonstrated that artificial congregation sites (e.g. mineral licks) where cervid saliva, urine, and feces are aggregated create prion reservoirs in local soils and water sources that can facilitate indirect transmission of infectious prions to cervids. Experimental evidence has also documented that CWD can be transmitted indirectly to susceptible animals via environments contaminated by excreta (i.e., urine or feces) and decomposing carcasses of CWD-infected cervids (Miller et al., 2004). Given this, current CWD management best management practices (Gillin and Mawdsley, 2018; Fischer and Dunfee, 2022) recommend that cervid baiting and feeding be prohibited following the discovery of CWD to avoid creating foci of contamination to which cervids are drawn, as well as moratoriums on the movement of live, captive cervids and high-risk, hunter-harvested cervid parts from CWD foci. Although logical and supported epidemiologically, specific assessments of the effectiveness of the foregoing measures have not been reported.

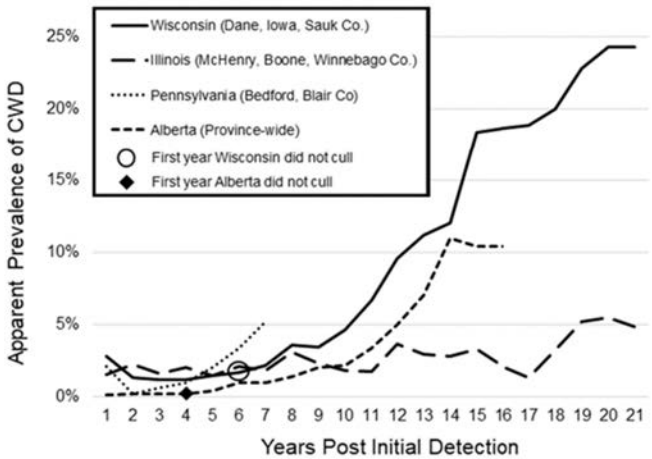


FIGURE 6.1 The annual apparent prevalence of CWD after initial detection as reported by four different agencies. The circle and diamond indicate when Alberta and Wisconsin stopped their culling programs. Illinois (long dash) has culled annually since detection and prevalence rates have remained relatively low. Pennsylvania (dots) did not implement long-term or at large scale sculling. Geographic areas for which prevalence is calculated is in parentheses following the name of each region
SOURCE: Thompson et al., 2023.

In addition to the practices described above, nearly all of the previously cited published reviews of free-ranging cervid CWD management successes, failures, and best management practices emphasize the critical importance of integrating well-constructed and long-term efforts to engage and educate interested and affected parties into an agency's overall CWD management approach. A review of these documents also illustrates that support from interested parties for CWD management tactics, regulations, and policies is much more difficult to secure after the disease is found if that support was not already in place (at least partially) as a product of a pre-detection CWD response plan drafting process (see previous section on pre-detection activities). Given the numerous factors (cultural relevance, political incentives, recreational values, economic impacts) that influence the support of constituents and interested parties for CWD management strategies described in the citations above, long-term viability (and thereby effectiveness) of control efforts appears to benefit from investment in long-term public education and engagement efforts that dovetail with proposed disease control efforts.

Box 6.4 provides a toolbox of sound CWD control strategies that have been or will be described in this chapter, and Table 6.2 presents published examples where the apparent effects of management strategies have been quantified *in situ* in free-ranging cervids. As noted elsewhere in this report, the ultimate impacts of CWD management strategies can be difficult to measure. However, the observed effects documented in those examples (Table 6.2**)** illustrate that levels of CWD control can be achieved and that experimental approaches to future CWD management are needed to allow for more rigorous assessments and comparisons of the apparent effects of various CWD management techniques employed by management authorities (Conner et al., 2007; Western Association of Fish and Wildlife Agencies, 2017).

Adaptive Responses for Sustained Control

Experience to date dictates that initial responses to CWD outbreaks will need to be modified over time as circumstances change, or as new or better information becomes available to responsible resource managers (see Box 6.2 for a description of adaptive management). As CWD and its management progress over time, geographic area, impacted species, and agencies with jurisdiction over free-ranging cervid health will experience a plethora of emerging factors that challenge adaptation. These include, but are not limited to, political opposition to disease management interventions that span multiple administrations, limited sustained funding needed to implement management strategies at time scales required to affect population level disease control, opposition by interested groups (e.g., hunters, captive cervid facility representatives, and conservation NGO's), and limited CWD testing capacity and cervid carcass disposal options (Thompson et al., 2023).

Given the above challenges that represent the complex biological, social, political, and temporal variables that can impact the efficacy of CWD control efforts within free-ranging cervid populations, no standard or uniform management recommendations exist that can be applied to all CWD-affected areas, cervid populations, or disease management timelines. Therefore, an adaptive approach that incorporates the previously referenced known best management practices and constructs current and prospective management strategies as experiments has been suggested as likely the most effective approach to successful and long-term CWD management (Conner et al., 2007; Western Association of Fish and Wildlife Agencies, 2017). Although tailored to western jurisdictions, landscapes, and cervid species, the approach can likely be applied to other regions of the United States. Considering the uneven effectiveness of past actions to control CWD in free-ranging cervid populations, the broader embrace of testable management actions, data collection and evaluation, and coordinated, adaptive disease management planning as proposed in the Western Association of Fish and Wildlife Agencies (2017) recommendations offers a viable framework for refining future efforts.

BOX 6.4
A Toolbox of CWD Control Strategies

Collective experience addressing CWD, other prion diseases of livestock, and other infectious diseases of livestock and wildlife provide a foundation for effective CWD control at local and national levels. Broader implementation of these principles and approaches could aid in curtailing CWD in parallel with the development of new knowledge and tools. This "toolbox" summarizes some of the principles and strategies for CWD control already available and described in Chapter 4 and this chapter.

General principles and strategies for control

- Situational awareness and assessment incorporating knowledge of risk factors, prevalence, host and environmental factors, and animal movements;
- Emphasis on and actions supporting prevention;
- Reduction or elimination of human-facilitated spread of infected cervids and other sources of infection (e.g., tissues and products from infected cervids;
- Consideration and elimination or reduction of potential sources of indirect exposures.
- Early detection and response to minimize environmental prion build-up;
- Need for realistic and plausible control goals that incorporate socioeconomic, political, and cultural dimensions in a collaborative and adaptive framework; and
- Preemptive public messaging and education on CWD and prospects for control.

Principles relevant to control in captive cervids

- Enhanced biosecurity principles, including certified animal sources;
- Quarantine principles applied to infected herds; and
- Intra- and interstate regulations and policies to support the foregoing.

Principles relevant to control in free-ranging cervids

- Surveillance and monitoring sufficient to detect outbreaks and assess responses;
- Established, long-term response plan that affords flexibility and use of multiple tools based on local conditions and circumstances;
- Use of culling or harvest to reduce host abundance and sources of infection; and
- Regulation or banning of baiting and feeding.

NATURAL AND ARTIFICIAL SELECTION

Studies in free-ranging deer and elk herds are beginning to uncover evidence of natural selection for less susceptible *PRNP* genotypes on a local level in areas of relatively high CWD prevalence (Monello et al., 2017; Chafin et al., 2020; Ketz et al., 2021), although none so far have considered genome-wide associations with susceptibility that have recently been uncovered. As discussed in Chapter 2, prion infections are inextricably linked to the host's prion protein, encoded by the prion gene—*PRNP*. Hosts lacking a functional prion gene, either naturally or through various means of genetic manipulation, are inherently resistant to prion infection and incapable of developing disease with even high-dose experimental exposure (Büeler et al., 1993; Richt et al., 2006; Yu et al., 2009; Benestad et al., 2012). Alternatively, hosts with natural variations in the prion gene (e.g., sheep as described in Chapter 2) are highly resistant to infection following either natural or experimental routes of exposure—a finding that has led to the near-eradication of scrapie from North America and other sheep producing countries through the selective breeding of individuals carrying what are considered conventional scrapie-resistant *PRNP* genotypes (USDA Animal and Plant Health Inspection Service. 2023; Melchior et al., 2010). Unlike as observed with sheep, there have not been any naturally occurring variants of the *PRNP* gene in any cervid species that are, alone, highly protective against CWD infection. Amino acid variations are

present in many cervid species, often linked to altered susceptibility or delayed disease progression (see Chapter 2). Current research seeks to determine whether *PRNP* variations in white-tailed deer are sufficient to prevent infection with natural exposure (Haley et al., 2021a), with a second study highlighting the significant role that genes beyond *PRNP* have in preventing infection in captive cervids (Seabury, Lockwood, and Nichols, 2022).

Investigating the potential for artificial selection to aide with CWD management in captive cervids has, to date, been challenging as most captive herds with confirmed cases of CWD are immediately placed under quarantine, making it difficult to modulate herd genetics through the addition of new breeding males and females**.** Box 6.5 provides a description of the role genetic selection might have in the management of CWD in captive herds. Ultimately, most of these herds are depopulated. One ongoing project is assessing the feasibility of CWD management through selective breeding of captive white-tailed deer with less-susceptible *PRNP* alleles on a property with historically high disease prevalence. At the onset of the study, the highly susceptible 96G allele (see Chapter 2) made up over 90 percent of the alleles present on the farm, and CWD prevalence ranged from 20 to 60 percent; individual homozygous (i.e., having two identical versions of the same gene) for the 96G allele made up a disproportionate number of positive animals, as reported previously (Haley et al., 2021a; Ketz et al., 2021). Over the first phase of the project, *PRNP* allele frequencies were shifted such that by the seventh year of selective breeding, the 96G allele made up less than 15 percent of the alleles present, with the 95H and 96S alleles making up much of the remaining alleles (Haley et al., 2021b; Haley, unpublished data).

BOX 6.5
The Role of Genetic Selection in Managing CWD in Cervids

For over two decades, artificial selection has been used with great success in the management of scrapie in sheep across North America and Europe, where this disease has been present for decades if not a century or more (Hagenaars, et al., 2010; See: https://www.aphis.usda.gov/sites/default/files/scrapie-quarterly-report-june-2024.pdf). Selection in sheep has solely focused on the *PRNP* gene, with the goal of increasing the frequency of sheep carrying the scrapie-resistant "ARR" genotype in domestic flocks (Goldmann, Hunter, and Foster, 1990). Although this approach has significantly reduced the number of scrapie positive animals to the point of near-eradication, ARR sheep are not completely resistant to scrapie infection (Lacroux, et al., 2017; Groschup et al., 2007). This finding highlights two important factors in managing TSEs in animals: (1) the polygenic nature of prion diseases (Seabury, Lockwood, and Nichols, 2022; Seabury et al., 2020)—not unlike most, if not all infectious diseases affecting livestock (e.g., Koets et al., 2000; Gul et al., 2022), humans (e.g., Schmidt et al., 2022), and even plants (e.g., Demirjian et al., 2022) and (2) the utility of selective processes in effectively managing TSEs despite the demonstrable susceptibility of animals considered "resistant" to infection.

Until recently, understanding of susceptibility and resistance to CWD in cervids has also focused on the *PRNP* gene—with animals carrying certain *PRNP* alleles (e.g., 96S) having a significantly lower risk of being CWD positive compared to their "wild type" counterparts (Keane et al., 2008a). The continued finding of both natural and experimental infections in these animals, despite their apparently reduced susceptibility, has understandably given members of the scientific community and regulatory agencies pause concerning the usefulness of selection in managing CWD and a call for more research into validation of the approach (USAHA Committee on Farmed Cervidae et al., 2024). Recent exploration of genome-wide markers of susceptibility in white-tailed deer, however, has created a more holistic picture of what it means to be susceptible or resistant to CWD (Seabury, Lockwood, and Nichols, 2022; Seabury, 2020). These studies have led to the methodical development of genomic estimated breeding values (GEBVs), which consider thousands of potential markers of susceptibility, that more accurately predict an animal's likelihood of developing the disease than the *PRNP* gene alone. Studies selecting for animals with GEBVs that provide a level of protection against CWD infection similar to those achieved with ARR sheep and scrapie are underway, with the hope that selection will prove as effective in managing the disease in farmed cervids as it has in the near elimination of scrapie in domestic sheep flocks.

Using crude markers of fitness, there have been no observed adverse effects in reproductive rates or the subjective health of offspring born with less-susceptible *PRNP* genotypes in the study's initial phase. The project's second phase will assess the impact of changing *PRNP* allele frequencies on disease prevalence, with some early evidence of a meaningful reduction in disease occurrence (Haley et al., 2021b).

Although the role that the *PRNP* gene in prion infections is well understood, other genes or loci must have a role throughout disease pathogenesis, from prion uptake in the gastrointestinal tract, to hematogenous, lymphoreticular, and peripheral nervous system trafficking, and ultimately amyloid clearance from the periphery and central nervous system. Recent and ongoing genome-wide association studies (GWAS) in both white-tailed deer and elk have identified a suite of polymorphisms strongly associated with infection status, many of which overlap with those associated with an increased risk of human Creutzfeldt-Jakob disease and other diseases (Seabury, Lockwood, and Nichols, 2022; Seabury et al., 2020). Collectively these polymorphisms can be used to assign individual animals with genomic estimated breeding values (GEBV), guiding selection of ideal breeding candidates for management through enhanced resistance to disease (Stear et al., 2012). This process has been used in a range of species to advance livestock health—including improved nematode resistance in sheep to viral resistance in captive sea bass (Palaiokostas, et al., 2018; Hayward, 2022). With aggressive management, including targeted culling of animals with poor GEBV and intensive breeding with high GEBV animals, significant improvements in herd-level GEBVs were seen in 2-3 years (C. Seabury, communication to the committee, December 14, 2023). Field studies in captive white-tailed deer employing GEBV-guided selective breeding are underway, and though objective data is not yet available, they have the potential to show additional gains in resistance beyond those currently expected from a focus on the *PRNP* gene alone.

While selective breeding may have the potential for facilitating CWD prevention and management in captive cervids, it may be inconsistent with the current paradigms for management of free-ranging deer and elk species. This paradigm does not preclude, however, the ability to observe natural changes in gene frequencies in CWD-affected free-ranging cervids over time. To date, these studies have focused exclusively on the frequencies of *PRNP* polymorphisms in high prevalence areas, though future studies employing GWAS in free-ranging cervids where CWD occurs may provide additional support for disease resistance approaches in captive cervids. In white-tailed deer, for example, a retrospective guided modeling study predicted that the frequency of the 96S allele in free-ranging animals, which typically makes 25 percent or less of the alleles in free-ranging populations (Johnson et al., 2006a; Brandt et al., 2015; Raudabaugh et al., 2022), would become dominant in an evolutionarily short period due to CWD selective pressure (Robinson et al., 2012; Ketz et al., 2021). Two separate modeling studies in elk, using regional *PRNP* gene frequency data, have also found that polymorphisms in this gene may help stabilize affected populations, with the potential to mitigate risks for local extirpation (Monello et al., 2017; Fameli, et al., 2022). Finally, studies in mule deer have reported evidence of selective pressure against the highly susceptible 225S genotype, with an increasing frequency of the less-susceptible 225F allele over just a couple of decades in CWD-affected populations—more so in herds with higher prevalence (Jewell et al., 2005; LaCava et al., 2021; Fisher et al., 2022). However, evidence of subjective behavioral and health abnormalities in 225F homozygous mule deer in captivity (Wolfe et al., 2014) combined with observed scarcity of 225FF individuals in the wild—even in the face of abundant selective pressure from CWD (LaCava et al., 2021; Fisher et al., 2022)—point to the further potential of a balancing equilibrium between CWD and underlying 225F fitness-based selection pressure. In addition to host fitness, it is important to note that many of these studies do not consider the risks of newly developed strains of CWD, particularly those that might preferentially infect cervids with *PRNP* genotypes presently considered less susceptible to infection (Velásquez et al., 2015), as well as the downstream effects of infected cervids with extended incubation times shedding infectious prion in the environment for prolonged periods (Cheng et al., 2016).

Although past studies on CWD-driven selective processes in cervids has almost exclusively focused on the *PRNP* gene, future studies considering genome-wide polymorphic genes and loci may further inform our understanding of disease susceptibility. Patience should be afforded towards studies

investigating either selective process, as increasing disease resistance may take several years to manifest in the case of captive cervids, decades perhaps in free-ranging cervids (e.g., LaCava et al., 2021). It remains to be seen, however, if genetic changes over time—through either selective breeding or natural selection—will affect duration of prion shedding or susceptibility to novel CWD strains. In addition to these concerns, studies should also consider factors such as host fitness (e.g., reproductive rates and offspring health), disease pathogenesis, and importantly the effectiveness of current diagnostic protocols in identifying infected individuals.

VACCINES

The development of vaccines for prion diseases like chronic wasting disease is challenging because the infectious prions are considered "self-proteins" (i.e., produced by the host itself), and thus incapable of eliciting a detectable adaptive immune response (generation of an antibody response)[12] (Pilon et al., 2013; Zabel and Avery, 2015; Wood et al., 2018). To overcome this problem, research groups are applying novel approaches to vaccine development. An early vaccine using antibodies generated against a short part of the prion protein (the "YYR epitope") has, to date, had limited success (Tashuck et al., 2014; Wood et al., 2018). Although trials in mice found the approach both safe and effective at generating an immune response, a field trial of the YYR vaccine in elk kept in CWD-contaminated paddocks found that vaccinated animals had shorter incubation periods than unvaccinated animals (Wood et al., 2018). There were multiple potential explanations for this, including the possibility that the antibodies to the vaccine induced misfolding of PrP^{C}, or antibody binding of PrP^{Sc} in the gut resulted in increased uptake of infectious prions, among others (Wood et al., 2018).

Another more promising approach warranting further investigation used constructs composed of paired PrP^{C} molecules ("dimeric PrP^{C}") expressed in bacteria (i.e., "recombinant" prion proteins, a process similar to components of the RT-QuIC diagnostic assay [Chapter 4]). The antibodies generated were specific for PrP^{C} and provide protection against prion infection in mouse models of CWD (Abdelaziz et al., 2017; Abdelaziz et al., 2018). The PrP^{C}-specific vaccine targeted PrP^{C} outside the brain, using aggregation-prone recombinant prion proteins for overcoming self-tolerance (Abdelaziz et al., 2017; Gilch et al., 2003; Kaiser-Schulz et al., 2007). Proof-of-concept in rodent and reindeer models demonstrated that tolerance to the PrP "self-proteins" can be overcome by this approach, resulting in detectable humoral and cellular immune responses without adverse side effects (such as autoimmune disease) and significant protection in CWD challenge models (Abdelaziz et al., 2018).

A third approach incorporated PrP^{Sc}-relevant epitopes onto the surface of fungal proteins (specifically Het-S, which also undergoes fibrillization [Wasmer et al, 2008]). The antibodies generated by this approach are specific to $PrP^{Sc,}$ and are hypothesized to interfere with PrP^{Sc}:PrP^{C} interactions, thereby blocking conversion. A similar approach provided protection in mouse models of Parkinson's disease (Pesch et al., 2024). Efficacy of the vaccine against cervid PrP^{Sc} needs to be evaluated.

A significant amount of research with the various vaccine candidates is needed, including extensive testing in captive cervids followed by pilot studies in some well-studied free-ranging cervid populations, focusing on efficacy, impact on shedding as well as duration of protection. Ongoing experiments in experimental infected captive white-tailed deer and in naturally infected free-ranging elk will provide objective data on these vaccine candidates. In the short term, these may prove most useful in captive herds. Long term goals would include the development of oral vaccines, analogous to those used for rabies (Slate et al., 2009), for vaccination of free-ranging populations. In addition, perspectives and acceptance by the broad array of interested parties and groups will affect the success of any potential vaccine applied to free-ranging cervids. Social science/human dimensions investigation prior to the delivery of any vaccine is essential. Barriers to vaccination also exist, especially in free-ranging cervids, notably given the potential need for repeated vaccination in the face of chronic exposure, challenges

[12] See https://www.cancer.gov/publications/dictionaries/cancer-terms/def/adaptive-immunity (accessed October 26, 2024).

associated with oral uptake of a vaccine, and uncertainties about the impact of the vaccines on shedding of CWD prions as well as the potential protection against a range of different CWD strains.

Without better knowledge of deer and prion biology, current vaccine development strategies may not be optimally utilized. While the development of vaccines for CWD are an important endeavor—ultimately helping guide strategies for human protein misfolding disorders **(**Kwan et al., 2020)—the same complexities concerning the potential effectiveness of genetic selection also apply. For example, while prolonged survival in mouse models following vaccination and subsequent infection with CWD has been observed (Abdelaziz et al., 2018), it is unknown whether vaccination with any of the proposed vaccine candidates would prevent CWD transmission or reduces prion shedding. Current and future vaccination strategies also need to consider their effectiveness following challenge with naturally occurring levels of CWD prions representing multiple CWD strains, and whether vaccination may impact the accuracy of available diagnostic tests (see e.g., John Pasick, 2004).

ENVIRONMENTAL CONTROL

Stopping or slowing the spread of CWD will also be dependent on decontamination of the environment. Although it is not known how long CWD prions will persist in soil matrices and other soil components (e.g., nasal bot larvae maturation in soil [see Chapter 3]; Soto et al., 2024), on plants, and on other fomites, a variety of studies suggest CWD prion persistence is variable, depending on environmental conditions and soil types. Several different approaches have been tested or are being developed. In South Korea, a contaminated farm area was extensively treated to attempt to remove all environmental sources of CWD. Topsoil was removed, the landscape then treated with hydroxide and then the area remained unoccupied for several years (Sohn, 2018). The efficacy of this treatment is unknown. The extensive nature of the approach would, however, be all but impossible to reproduce on the scale needed to decontaminate all foci in the United States, and thus more practically limited to farmed cervid facilities.

Several groups have suggested that humic acid (HA), a naturally occurring component of some soils, has anti-prion properties (Giachin et al., 2014; Kuznetsova et al., 2018). HA comprises much of the organic matter in the upper horizon of soils in many regions where CWD occurs and is also a component of many fertilizers. Humic acids are comprised of polymeric mixtures of weak aliphatic and aromatic organic acids, encompassing a wide variety of organic structures. In the research lab, using enriched PrP^{Sc} preparations and purified humic acid, the PrP^{Sc} appears to be degraded, and infectivity reduced, when CWD samples are treated with HA at concentrations commonly found in a variety of soils (Kuznetsova et al., 2018). It has not, however, been demonstrated that HA has efficacy against soil-bound prions as interactions between prions and soil may increase the resistance of CWD prions to degradation.

Numerous studies over the past few decades highlighted the ability of high temperatures to inactive prions. Most studies involved incineration and very high titers of CWD (greater than 10^9 infectious units per gram of tissue). Incineration of prion-infected tissues at 600°C did not completely inactivate the infectious prions despite ashing (i.e., removing organics from) the material (Brown et al., 2004). However, at temperatures of 1,000°C, no infectivity remained. Inactivation of prions at high temperatures led to suggestions that wildfires could perhaps decrease the environmental load of CWD, particularly when prion levels in the environment are low. Although it is unlikely that most wildfires would attain temperatures sufficiently high to inactive high titers of CWD, removal of infected plants and, perhaps, surface soils containing prions could reduce the amount of CWD in the environment (Zabel and Ortega, 2017). Given the practice of prescribed burning for other landscape management purposes, further research on the impact of prescribed or natural wildfires on environmental prion remediation is warranted.

Given the combination of the long pre-clinical phases of disease, coupled with environmental contamination that may play a role in ongoing transmission, a multi-pronged approach to mitigating disease is requisite. Currently, the efficacy of these potential environmental mitigation strategies has not been fully determined and at present, none are recommended for use by cervid farmers or wildlife managers (USDA APHIS, 2019; Gillin and Mawdsley 2018). Like CWD vaccine development, methods

for decontamination of the environment are not imminent. This is primarily due to the difficulty of detecting and quantifying CWD prion load in the environment; the knowledge of how much CWD prion must be removed to decrease the likelihood of transmission and the soil/vegetation-type variation in avidity of prion binding.

EFFECTIVENESS OF PUBLIC MESSAGING IN CWD MANAGEMENT

A sometimes overlooked and often undervalued consideration for management of complex problems like CWD is effective public messaging—including all forms of media, and social media (Merchant, South, and Lurie, 2021). With the appearance of COVID-19 in late 2019 and early 2020, the world experienced an unmistakably new era of mis- and disinformation (e.g., the generation and amplification of information that is either unintentionally or intentionally false and misleading which undermines the goals of disease management and, ultimately, negatively impacts public health (Ferreira Caceres et al., 2022). Many of the same public health hurdles faced during the COVID-19 pandemic and subsequent "infodemic,"[13] including conveyance of information on the origin and nature of the agent itself, its importance to human, animal, and environmental health, and the need for collaborative management strategies, overlap those seen for CWD. Efforts have been made by several public, private, and governmental groups around the country to provide accurate information and combat misinformation and disinformation from primarily social media sources, with mixed success as discussed below. Fortunately, the global experience of information processing during the COVID-19 pandemic has provided businesses, health experts, and governments with valuable insights into how to best approach public messaging for agents like CWD (Smith et al., 2023).

Social media plays perhaps the most significant role in the dissemination of misinformation and disinformation for diseases like CWD, typically having a more rapid and far-reaching distribution than factual information provided by health professionals (Muhammed and Matthew, 2022; Allen, 2022[14]). Top-down disinformation, arising from various "influencers," including politicians and celebrities, generates the majority of the social media engagement, though may only make up a small percentage of all the misleading claims that might be found (Gisondi et al., 2022).

Despite this, social media also has a key role in the distribution of accurate and timely information. Many state agencies have built social media profiles on a range of different platforms, from Facebook to YouTube, that provide up to date information on CWD and offer citizens the opportunity to participate in management efforts (see, for example, the Wisconsin Department of Natural Resources YouTube Channel[15] and New York State Department of Environmental Conservation Facebook Page[16]). Both public and private enterprises, such as the National Deer Association and Safari Club International, have assembled educational webpages and gotten involved in grassroots management approaches that incorporate social media (National Deer Association;[17] Safari Club International CWD Strategy[18]). Recruitment of recognizable and relatable experts in the hunting and cervid health fields is an important avenue to pursue to maximize impacts in social media; however, these figures may hesitate to wade into discussions that have moved beyond scientific debate into politicization (N. Pinizzotto, National Deer Association, personal communication, January 19, 2024). Additional steps taken by health experts on social media, including accuracy prompts and debunk interventions (Smith et al., 2023), are also necessary to promote public understanding of the disease itself as well as management options and goals.

[13] The World Health Organization describes infodemics here: https://www.who.int/health-topics/infodemic#tab=tab_1 (accessed October 4, 2024).

[14] See https://integrityinstitute.org/blog/misinformation-amplification-tracking-dashboard.

[15] See https://www.youtube.com/@WIDNRTV (accessed August 27, 2024).

[16] See https://www.facebook.com/NYSDEC (accessed August 27, 2024).

[17] See https://deerassociation.com/cwd/ (accessed August 27, 2024).

[18] See https://safariclub.org/scif-cwd-strategy/ (accessed August 27, 2024).

However, at present little is known about the effectiveness of many of these public messaging interventions. See Chapter 7 for more on the social dimensions of CWD.

MONITORING CWD CONTROL EFFORTS

As with most infectious diseases affecting livestock and wildlife, the effectiveness of CWD management and control programs needs to be evaluated based on their success in reaching target goals set by the entities initiating the programs. These goals often are not clearly stated and are then assumed to include the prevention of disease incursion, a stabilization of disease prevalence, or outright disease elimination, which may not be realistic. Evaluation of management plans could be more effective if target management goals and the sound scientific justification for those targets are defined. Theoretically, the availability of robust surveillance data and more precise animal inventories and tracking of captive cervids makes control outcomes more practically quantified and thus more quickly refined than control measures applied to free-ranging cervids.

The HCP is well positioned to provide yearly occurrence rates and geographic distribution of CWD among cervid farming operations, for example, and goals may be to reduce yearly case incidence, to more rapidly identify positive cases, or to prevent disease incursion into unexposed areas. On the other hand, within an individual herd in which a management program has been implemented, target goals might include reduction in prevalence to below a specific threshold within a given time frame, and then stabilization or continued reduction beyond that period. With well-described targets, these plans may be continuously evaluated, modified, or revised as necessary to further enhance and inform future management and control efforts.

The main elements for assessing the effectiveness of interventions to control or prevent CWD in free-ranging populations are analogous to those described for assessments in captive settings. Establishing a biologically relevant and biologically representative spatial unit for making these kinds of assessments is an important added consideration. As noted, efforts to manage CWD in the wild are never fully under human control, are inherently complicated by societal and political constraints, and require an extended time to observe and quantify responses. An adaptive approach that incorporates and compares current and prospective management strategies as experiments has been embraced by responsible agencies as a viable framework for developing effective long-term CWD management.

7
The Human and Socioeconomic Dimensions of CWD

Chapter Highlights

- Complex human dimensions—like biological factors—are influential in the transmission, spread, and management of CWD. Lack of understanding of the social, economic, and cultural aspects (i.e., human dimensions) of CWD is a significant barrier to the management of the disease.
- Interested and affected parties hold different views, values, beliefs, and economic interests and their behaviors and decision-making vary greatly. At a time when collaboration and cooperation are critical, the division among interested and affected parties can result in a lack of participation, compliance, and collective support for CWD actions and programs.
- A lack of quality economic data can lead to undervaluing the impacts of CWD and the inability to assess key resources, management and control strategies. However, there is evidence to conclude that CWD is an expensive disease and is becoming increasingly costly and consequential in both captive and free-ranging cervids.
- To better understand and leverage human dimensions in managing and controlling CWD, the addition of human dimensions experts and researchers—including, sociologists, economists, behaviorists, anthropologists, and others—is needed. Managing CWD requires the integration of many disciplines and experts to co-create a new convergence in thinking, innovations, and strategies.

Chronic wasting disease (CWD) threatens a national resource and entire ecosystems that are valued, relied upon, and enjoyed by Indigenous communities, hunters, cultural and ecological conservationists, and recreationalists across North America. Although biomedical and epidemiological research have contributed to general knowledge about CWD, human attitudes, values, beliefs, and behaviors also influence how CWD is understood and responded to. Animal and wildlife health and diseases such as CWD cannot be managed effectively without understanding the people and policies that affect or are affected by wildlife health (Stephens, 2022). The social dimensions of CWD, including the values, perspectives, preferences, and behaviors of different affected parties, rightsholders, and agencies need to be identified and understood. The multiple governmental agencies responsible for managing or controlling some aspect of CWD, are also governed by different interests, responsibilities, or priorities. For example, the authority to protect and manage cervid species held in public trust lies with state, provincial, and territorial governmental wildlife and environmental authorities. However, state and federal agricultural departments have primary responsibilities for regulating captive cervids in most states.

Human dimensions refer to how and why people value natural resources, their priorities regarding management of those resources, and the impacts of official decisions and control strategies applied to people. This chapter addresses aspects of human dimensions associated with CWD, the complexities of understanding and management of CWD as discussed in previous chapters, and the economic impacts of CWD. Box 7.1 provides some insights on the different dimensions impacted by the detection of CWD in a state. Human dimensions encompass multiple disciplines such as psychology, sociology, and economics, and thus refer to all social considerations in a variety of fields including those of fisheries and wildlife (Decker et al. 2012). Economic theory and application are critical for understanding both the monetary and utility-based incentives and challenges associated with CWD prevention, surveillance, management, and the realized costs generated by this disease. And finally, while tribal communities may be socially and economically impacted by CWD in many of the same ways as non-tribal individuals or jurisdictions, deep-rooted cultural values and practices exert an additional dimension of complexity.

BOX 7.1
Socio-Economic Impacts of the Introduction of CWD in New York State

In late March of 2005, a CWD-positive white-tailed deer was reported in a small private deer herd in Oneida County, New York. The case was identified through New York's mandatory testing program for captive cervids, which began in 2002 (Garruto et al., 2008; Schuler et al., 2022). Subsequent trace-outs identified a second positive deer in a separate, nearby captive herd (Brown et al., 2006). A swift quarantine and depopulation of the captive herds led to the discovery of 3 additional positive animals in the first herd (Brown et al., 2006).

The state's Department of Environmental Conservation (DEC) enacted a swift plan to surveil free-ranging deer in a containment area with a radius of 10 miles from the initial cases in farmed deer (New York State Department of Environmental Conservation [DEC], New York State Department of Agriculture and Markets [DAM], and Cornell University College of Veterinary Medicine Wildlife Health, 2018). Outreach focused on educating and informing the public and seeking landowner permission to facilitate wild herd reduction and testing efforts (Brown et al., 2006). With the establishment of a local field laboratory, over 300 wild white-tailed deer were harvested and tested over the ensuing month, with two positive cases identified in wild deer (Garruto et al., 2008). Several emergency regulations were then enacted for the containment area, including mandatory hunter check stations, a ban on movement of intact carcasses, and additional requirements for taxidermists in the area. More than 7000 deer were killed (mostly by hunters) and tested over the next five years from the containment area, with no additional cases identified; the containment area was subsequently decommissioned in 2010 (Brown et al., 2006; Shuler et al., 2022). The DEC estimates that between public outreach, targeted harvesting efforts, and surveillance, the incursion cost the state roughly $1 million (Shuler et al., 2022).

A 2019 New York DEC-sponsored study found that the large majority of hunters in the state had concerns about consuming venison from CWD-positive sources and agreed that disease spread would negatively impact hunting opportunities in the state, although engagement in preventative recommendations provided by the DEC—including proper disposal of carcasses in landfills and avoiding the use of natural scent lures—often went unheeded (Siemer, Lauber, and Stedman, 2020). The authors of that study suggested that this may be due to hunters' low perception of vulnerability to any negative outcomes, and that their actions may have limited effect in preventing further introductions of CWD into the state.

SOCIAL DIMENSIONS OF CWD

Numerous individuals and interest groups hold diverse and opposing views regarding many aspects of CWD and its management. The motivations of landowners, alone, regarding CWD surveillance participation, for example, are diverse and nuanced (Rubino and Serenari, 2022; Landon et al., 2022). Individuals and interest groups make up an intricate human ecosystem that influences public participation in interventions, decision-making by officials, and preferences by the interest groups themselves (Ufer et al. 2023). Vaske (2010) conducted a literature review which resulted in seven findings that outline attitudes, behaviors, and beliefs among various interest groups regarding CWD based on human dimensions research methodologies below, along with examples of the individual studies that informed the specific conclusions (Vaske, 2010; p166):

1. *Hunters vary in their behavioral response to CWD* (e.g., Needham, Vaske, and Manfredo, 2004; Brown et al., 2006; Needham and Vaske, 2006; Vaske and Lyon, 2011) (see Box 7.2)
2. *CWD affects a variety of stakeholders* (e.g., Brown et al., 2006; Anderson, Frosch, and Outlaw, 2007a; Stafford et al., 2007; Arnot et al., 2009)
3. *Perceived human health risks can influence behavior* (e.g., Gigliotti, 2004; Miller, 2004; Gore et al., 2009; Sjöberg, 2000a, 2000b; Heberlein, 2004; Cooney, 2008)
4. *Perceived health risks and agency trust influence acceptance of management actions* (e.g., Needham and Vaske, 2006; Stafford et al., 2007; Brown et al., 2006; Miller, 2003; Cooney and Holsman, 2010)
5. *Stakeholder knowledge about CWD varies* (e.g., Miller, 2003; Brown et al., 2006; Vaske et al., 2006; Anderson et al., 2010; Lischka, Shelton, and Buhnerkempe, 2010)

6. *CWD has social, economic, and managerial costs* (e.g., Bishop, 2004; Heberlein, 2004; Seidl and Koontz, 2004; Stubier et al., 2006)
7. *Not all management actions are equally acceptable and/or effective.* (e.g., Williams et al., 2002; Petchenik, 2006; Cooney and Holsman, 2010)

BOX 7.2
Hunter Behavioral Response to CWD

Generalizing hunter behaviors from the existing literature is challenging. There are pronounced differences in the responses of certain groups of hunters to CWD from others. If CWD has been recently discovered in a locale, or if prevalence rates are high, a proportion of hunters either stop hunting, change hunting locations, or change how or what they hunt (Vaske, 2010; Zimmer et al., 2012). Other hunters may not change their behaviors at all. Understanding behavioral responses of hunters to CWD is complicated by their perceptions toward risk related to CWD and their trust in the reporting agency from which they got their information (see Pattison-Williams et al., 2020 which includes a comprehensive review of related work for both the United States and Canada). As familiarity with the disease increases or there are no catastrophic collapses of cervid populations, the initial reductions in hunting or locational switching tends to dissipate (Bishop, 2004; Erickson et al., 2019).

Figure 1.2 introduced variables that, individually and collectively, determine disease outcomes. The factors are interconnected and constantly changing within different contexts of space and time. Critical insights of the disease are found at the interface of these domains and variables. Understanding the different human dimensions, the factors that drive them, and how they can be factored into solutions exemplifies the concept of convergence (see Box 7.3). Convergence models have been used to explain the complexities and relationships of factors that determine disease outcomes and can inform comprehension of the evolution of emerging infectious diseases including CWD (e.g., Institute of Medicine, 2003). In this model, the host-pathogen interactions are embedded and influenced by four domains including genetic and biological, environmental, ecological, and social-economic-political factors. Found within these domains are numerous drivers or factors that further influence and impact the dynamics of CWD, including human dimensions. Because human interests and behaviors drive many reactions, decision making, and response to CWD and management interventions, it is logical to assume that understanding and addressing the socio-economic factors and their impact on CWD disease process, progression, and control options will be important. The underlying differences in values that motivate action on behalf of free-ranging cervids or the captive cervid industry must be addressed (Organ et al., 2016).

BOX 7.3
Convergence Science and CWD

Convergence Science (e.g., NRC, 2014) can bring together experts, organizations, agencies, and private interests, to develop innovative approaches for problem solving, thinking, communicating, and connecting. A transdisciplinary approach is an essential component of convergence science that incorporates views and knowledge from the natural sciences (i.e., ecologists, geologists, biologists, chemists) human dimensions sciences (i.e., anthropologists, sociologists, economists, and psychologists) and health. This integration facilitates the sharing of information and alters the context for addressing important issues. Utilizing convergence science to control CWD could organize thinking, identify the relevant interested and affected parties/ sectors, introduce new incentives, and integrate new knowledge from multiple disciplines. Figure 1.2 demonstrates the complexity and interactions associated with CWD.

An effective CWD management plan requires research about and accounting for varied responses to uncertainties, risks, and knowledge gaps to build the trust necessary for policy deployment (e.g., Heberlein, 2004; Needham and Vaske, 2006; Vaske and Lyon, 2011; Heffelfinger et al., 2013). In the case of CWD, there is a need to understand the behavioral responses of different interest groups to both the disease itself and to policies surrounding disease management. This includes, for example, incentivizing the behavior changes that align with the social considerations of varied interest groups, and maintaining regulatory authority and consistency to evaluate CWD management options (Xie et al., 2023; Ufer et al., 2023). The consequences of not building trust among interested and affected parties in knowledge about the disease (i.e., the state of science) and in policies related to its management could result in additional or faster spread of the disease and threats to resources.

Collective and collaborative management planning has long been recognized as challenging especially when public policy decisions are involved (e.g., Rittel and Webber, 1973). This is true regarding CWD interventions given the different levels of awareness and understanding of CWD among interest groups, the lack of a single policy or intervention that will satisfy the interests of all interested groups, and any complacency that may exist about the disease among the public. Gaining the political support across local, state, and national governments is difficult even though CWD is an emerging, growing, and serious threat spreading into new geographic sites and populations. Without the political support and subsequent actions, CWD will almost certainly progress and spread into more states and become more difficult to control and more expensive to control.

A successful response to management of CWD is more likely through strategic collaborative efforts among the various groups interested in cervids (see Table 1.1) and CWD management (e.g., the CWD Task Force to be convened in response to the America's Conversation Enhancement Act [P.L. 116-118]) when such groups are committed to identifying and defining the problems to be solved and deciding on and using an analytic-deliberative process that is appropriate to those problems (e.g., NRC, 1996). Such approaches allow for the sharing and integration of knowledge; identifying objectives and metrics for their measure; identifying the priorities and rights of the individuals and groups affected; identifying of management alternatives, capacities, resources, and consequences of those management alternatives; and acknowledging and agreeing to the compromises that will be necessary to reach decisions (e.g., Keeney and Raiffa, 1993). Identifying the interest groups whose livelihoods and quality of lives may be affected by management decisions, recognizing that values and priorities will vary among and between groups (and among and between individuals in groups), and assimilating their knowledge into the decision process is an important part of the process.

There is also a need for effective communications—especially risk communications—that can help guide behavior and counter misinformation and misunderstandings (Leong and Decker, 2020). While understanding and managing wildlife populations is crucial, it differs from managing diseases within these populations. Both are needed but should not be confused as interventions evolve. See Box 7.4 for discussion of how views may differ among agencies.

ECONOMIC DIMENSIONS OF CWD

CWD poses risk to the abundance and health of cervid populations. In turn, this risk can disrupt the provisioning of highly valued ecosystem services and resulting in losses or costs to cervid farmers (and consumers of their goods and services), free-ranging cervid hunters (both recreational and for subsistence), outfitters, wildlife viewers, and conservationists. While changes in expenditures or costs are an important components of measuring loss, they do not capture the change in values induced by CWD. Changes in expenditures or costs are an important component of loss, but they do not capture the change in values induced by CWD.

BOX 7.4
Different Views Among Managing Agencies

Even among agencies with responsibility for management, there are different points of view regarding risk. Groups differing in authorities, philosophies and programmatic goals address different aspects of CWD. For example, wildlife managers may focus on conservation, population sustainability, and may view diseases as natural processes. Wildlife disease ecologists may have special interests in host-pathogen interactions often in the context of environmental and evolutionary changes. Disease management and control officials emphasize prevention, control and eradication goals that are closely aligned to traditional livestock control programs. Experts among these groups may represent different views, interests, and programmatic outcomes. Incorporating the evolutionary outcomes of environment-disease interactions will help to improve integration of ecological and conservation concepts into the study and management of wildlife diseases (Vander Wal, et al., 2014). This convergence of disciplines can be mutually beneficial and produce more unified and effective strategies to reduce the transmission and spread of CWD.

Measuring how values are generated is grounded in utility theory (i.e., the costs and benefits that determine choices that people make; Box 7.5 provides an explanation of utility theory). However, given the nature of the goods and ecosystem services affected by CWD, that some of those goods are traded in markets (such as captive cervid products), and that some are not (such as wildlife viewing), standard economic methods may be insufficient to consider all the consequences of CWD. Behavioral economics combines elements of economics and psychology to understand how and why individuals behave the way they do in the real world including making irrational decisions (Pesendorfer, 2006). Behaviors and decision-making are not always based on facts; those interested in and affected by data are subject to a variety of emotions, biases, and are further influenced by their environment and circumstances. Limited knowledge and misinformation can cause behaviors to emerge which may be difficult to alter, and subsequently lead to varied economic consequences.

Behavioral economics offers valuable tools for addressing the economic values different parties have for strategies and outcomes and can help measure and predict the support and participation of interested and affected parties. Behavior economic tools include contingent valuation, choice experiments, and experimental auctions that are commonly employed in assessing the preferences of interested and affected parties and understanding decision-making that could be applied (Freeman III, Herriges, and Kling, 2014). Incorporating such tools into understanding human dimensions can ultimately help to benefit the management and control of CWD (Ufer et al., 2022).

The next sections explore the economic dimensions of CWD and the associated economic values at risk. First, components of risk from an economic viewpoint are defined, then the text briefly describes how economic values are generated in general market settings, and then the text focuses on specific markets affected by CWD.

BOX 7.5
Utility Theory

Utility theory is based on the concept that people can make decisions based on ranking of their preferences and their resource constraints (Acemoglu, Laibson, and List, 2019). The preferences of interested and affected parties are reflected by different parties or groups having different utility functions (e.g., which reflect the welfare or satisfaction from a set of choices) resulting in differences in determination of their values. Utility theory can be used to determine the value or worth of goods or services and then to help explain how and why individuals make decisions and form preferences from a set of options or alternatives (e.g., Perloff, 2009). Because utility theory helps to understand and quantify the underlying value implied from an individual's choices, it can give important insights about who is affected by CWD, what interested and affected parties really care about, and ultimately their subsequent behaviors and decision-making.

Defining and Managing Economic Risk

There is a large economy directly and indirectly related to cervids including cervid farming, hunting, and recreation, and thus strong incentives or disincentives exist regarding CWD prevention and management in many of these cases (Organ et al., 2016). Many wildlife management agencies are funded through the purchase of hunting licenses. People and communities may count on the cervids for their livelihoods or food security, in addition to the cultural traditions or spiritual significance related to the treatment of the cervids, but it is not possible to accurately quantify the costs and economic impact of CWD at a national level. This inability precludes a true understanding of the economic risks and burdens of the disease which can result in the inability to conduct needed economic assessments or cost-benefit analyses of CWD control activities.

The economic definition of risk combines two elements: the likelihood of any event (e.g., an outbreak of CWD in a captive-cervid facility) and the severity of the outcome (harmful effects) if it is realized (Ehrlich and Becker, 1972). Understanding risk requires a knowledge of both elements (probabilities and outcomes) but a challenge in calculating risk is understanding and incorporating how human actions influence each. The likelihood of a localized CWD outbreak depends not only on forces outside of human control (e.g., climate, migration, and ecological conditions) but also on human behaviors that govern human interactions with wild and captive cervid populations (e.g., hunting, management of captive cervid facilities); trade of captive cervids and related practices and products; and on how people, business entities, and government agencies respond to and develop policies in response to the risk. The severity of economic outcomes depends on disruptions to business operations and trade, impacts to the values individual people place on cervids and the ecosystem services they support, and additional costs imposed on government agencies resulting from CWD and its management.

Utility theory (see Box 7.5) can be applied to measure outcomes, and in some cases, employs reported or market related data. However, in other cases additional information not reported or documented from market transactions is necessary. In addition, as humans act to mitigate a variety of personal risks that may or may not be related to CWD, they alter the likelihood and severity of a CWD outbreak through, for example, limiting or changing interactions with wildlife, changing production processes, or changing trading practices of captive cervids.

The ability of management agencies and officials to identify costs and benefits from a utility perspective enables them to maximize participation of interested and affected parties by aligning the parties' preferences and decision-making processes to acceptable strategies and actions responsive to those priorities. For example, the costs and benefits of management practices as perceived by hunters and non-consumptive wildlife enthusiasts (e.g., visitors to wild lands) will differ based on their utility functions, yet, both groups need to be participants and advocates of CWD control. Development of incentives to implement or comply with management decisions that are based on values of interested and affected parties may help to drive preferences and behavior (Ufer et al. 2023). To address the economic costs and benefits to a variety of interest groups associated with CWD, Ufer and colleagues (2023) reviewed numerous CWD management actions and their alignment with different interested and affected parties in a behavioral economics and utility theory framework. This means that observed behavior by an individual party was the focus, rather than stated intent or theoretical assumptions. The findings summarized human dimensions literature associated with a wide array of interested parties (both indirect and direct beneficiaries of cervids) as it pertained to behavioral economics and expected cost-benefit analyses for interested parties (Ufer et al., 2023). The associated need for regulatory agencies to incorporate behavioral economics and values of interested and affected parties in disease mitigation strategies appears to be a critical need for management and compliance of CWD (Ufer et al., 2023).

Market Values

Economic values generated in a market setting include, for example, those associated with the captive cervid industry and the related products and services they support. In such settings, both

consumers and producers of the products (farmers and related industries) have values that can be impacted by CWD—determined by measures such as lost consumer surplus and lost producer surplus. Consumer surplus (Silberberg, 1972) is the benefit to consumers of a market outcome and is generated whenever consumers pay less than their maximum willingness to pay for a unit of a good. As a hypothetical example, if a consumer was willing to pay $5000 for a captive bred cervid but pays a market price of $2000, the difference of $3000 is a measure of the benefit to that consumer. For all consumers, their maximum willingness to pay (demand) depends on individual preferences, resource constraints (such as their disposable income), and other drivers of demand. Together the number of units sold and the differences between the maximum willingness to pay and market price drive the magnitude of the benefit to consumers. The maximum an individual is willing to pay depends in an inverse fashion on how many units they are able to buy. If CWD results in fewer captive cervid farms or operations and reductions in inventories (or numbers) of captive cervids, reductions in available captive cervid products or services in the market as shown in Anderson and Chomphosy (2014), the prices consumers would be willing to pay per unit increases. Lost consumer surplus accounts for both the reduction in quantity bought and sold, and the change in willingness to pay.

Producer surplus (or the social cost to produce the good, Schmalensee 1971) is the difference between the market price and the minimum each seller (farmer or producer) is willing to accept to produce and supply the good. If the minimum a producer is willing to accept for a farmed cervid is $1000 and the market price they receive is $2000, the difference of $1000 is a measure of the benefit to the producer. Across all producers, the minimum each seller is willing to accept depends on the producer's technology and input costs. The greater the differences, and the more units produced and sold, the greater the producer surplus.

If CWD results in an increase in costs to producers (infrastructure expenditures such as double fences, operational expenditures such as alternative, higher cost feed sources and increased expenditures on veterinary services, trade restrictions and quarantines that lead to additional costs, costs imposed from herd depopulations) or reduces captive herds and the availability of goods and services (as considered for CWD in Canada; Arnot and others, 2009), the price producers would be willing to accept per unit would change. For many private entities and industries, that acceptable price falls, but this depends on industry specifics. Lost producer surplus considers both the reduced quantity supplied of products and services, and the change in the producers' minimum willingness to accept to produce and sell the product. The total loss in a specific market setting would be the sum of lost consumer surplus and lost producer surplus from CWD.

Values generated in a related market setting include those associated with hunting (Ufer et al., 2023). The hunting experience is a multi-dimensional good which has elements associated with expenditures such as those on hunting licenses, equipment and trip or access costs, and elements associated with the enjoyment of the hunting experience. A person's enjoyment of hunting drives expenditures on these "related goods." Changes in the abundance and health of a targeted free-ranging cervid population affects the quality of the hunting experience, altering the maximum willingness to pay for related goods, which changes the quantities of related goods purchased and consumer surplus from consumption of the related goods. If CWD reduces the quality of the hunting experience, there would be a reduction in the maximum willingness to pay for the related goods. Given the provisioning and prices of related goods will not change with the reduction in quality of the hunting experience, the lost consumer surplus from the reduction in consumption of related would measure the loss in value from CWD.

Values generated in a non-market setting include those associated with traditional subsistence strategies and with pure conservation values associated with cervids in the wild. These are generally termed "non-market values." Non-market values include those generated by traditional subsistence hunting and cultural practices, wildlife viewing, values associated with conservation of cervids in the wild, and the value from just knowing the cervids exist. Such values are challenging to quantify.

Economic Values Associated with Captive Cervids

The cervid farming industry in the United States comprises two types of operations, breeding operations, and breeding and hunting operations (Outlaw et al., 2017). Revenues of breeding operations come from sales of cervids and cervid products (e.g., urine used for hunting related products, velvet, and meat). Revenues from breeding and hunting operations come from sales of cervids, cervid products, and hunting on the property. Outlaw and others (2017) estimated the direct and indirect impact of the cervid industry (breeding and hunting) to be $7.9 billion (in 2017 dollars) and responsible for the employment of 56,320 individuals. In an earlier study Anderson, Frosch, and Outlaw (2007a) estimated the per farm direct and indirect financial contribution of the cervid industry to the economy as $260,000 per farm, and $2.3 billion (2007 $) in direct and indirect impacts.

CWD and its management affect captive-cervid farmers through animal mortality, increased costs of doing business (e.g., double fences, increased veterinary services), and with devaluations of farm herds as an asset through restrictions on business operations (e.g., trade restrictions, quarantines, and herd depopulations). If regulatory requirements result in farmers being unable to participate in the market or restrict the sale of their products, revenues decline, profits are lowered, and they may face a decline in economic welfare. CWD may also have indirect and less measurable impacts for captive-cervid farmers, for example, the emotional impacts on those deeply connected to their farms.

The economic value at risk to cervid farmers from CWD can be modeled as the reduction in welfare to the farmers relative to the non-CWD outcome. The welfare loss to farmers can be measured as the reduced profits and lost non-market value from direct and indirect impacts of CWD, which requires estimates of farmers' profit functions, knowledge of the relationship between price and quantity supplied in related markets, estimates of farmers' non-market values, and estimates of how CWD affects the operations and operational choices of and for cervid farms. The estimates of Outlaw and others (2017) and Anderson and others (2007a) are useful in scoping the problem but do not measure the reduction in individual farmer welfare for the CWD model outcome relative to the non-CWD model outcome. In an analysis noting the difficulty and data requirements in estimating the value at risk, Anderson and Chomphosy (2014) estimated the effect of state-wide presence of CWD on captive cervids and the number of farms in the United States from 2002 to 2007. Anderson and Chomphosy (2014) documented the number of captive cervid operations and herd sizes in state level inventories of captive cervids. They found that the 2007 presence of CWD in a state led to an over 50% reduction in statewide inventories of captive cervids (relative to a CWD free state). Most of that was due to a 45% reduction in the number of operations and operators exiting the market. There are insufficient data to allow projections to today.

Similar modeling conducted for Alberta and for all of Canada illustrates methods to estimate the captive cervid values at risk from CWD. Petigara and others (2011) used modeling similar to that of Anderson and others (2007a) and Outlaw and others (2017) to consider the effects of CWD as if it mirrored the consequences of the bovine spongiform encephalopathy (BSE) outbreak (i.e., resulting in a complete reduction in the demand for cervid industry products). See Appendix E for more information about the economic impacts of BSE and scrapie. They considered how consumption bans, changes in demand resulting from risk perceptions, and government trade restrictions would impact the cervid and related industries. Arnot and others (2009) modeled potential outcomes of CWD on cervid farms in Alberta resulting from (a) double fencing of farms, and (b) depopulation of cervid farms. Their results indicate that public subsidization of preventative strategies such as double fencing, which can be prohibitively expensive for individual farmers, are significantly more cost-effective use of public funds than indemnity payments for farm depopulations.

To help scope the problem for the United States, Chiavacci (2022) documents the number of cervid operations or farms and the number of captive cervids (inventories) by state in 2020. Appendix F provides several visualizations of those data from different perspectives to help the reader understand the numbers and locations of facilities. Some states such as Texas have both breeding and breeding and hunting operations. Little data exist on the scale of those operations apart from the facilities surveyed by Outlaw and others (2017).

Economic Value of Free-Ranging Cervids and Hunting

In the United States, in general, free-ranging cervids are held in public trust by state fish and wildlife agencies responsible for insuring healthy sustainable populations for the benefit of the public. On Tribal lands where state agencies do not have jurisdiction for wildlife management, that responsibility falls to Tribal natural resource agencies, and in some cases, there is co-management responsibility. In some cases, jurisdiction falls under federal authority (e.g. National Park Service, National Wildlife Refuge System). There are costs associated with those responsibilities, but there are also other values at risk from CWD including those associated with conservation, culture, and hunting. Hunters, in combination with anglers, boaters, and recreational shooters, contribute most directly to conservation of species and habitats via license sales and excise taxes on gear and equipment sales that, in aggregate, generate approximately 60 percent of state fish and wildlife management agency annual budgets (Thompson and Mason 2022; Association of Fish & Wildlife Agencies and the Arizona Game and Fish Department, 2017). Hunters, specifically, generated $824,973,807 (actual costs, not adjusted for inflated) in license and permit sales allocated to conservation budgets in 2016, $375,000,000 of which was provided by deer hunters who also contributed $20.9 billion to the United States gross domestic product that same year (Southwick Associates, 2018).

However, much of the value of free-ranging cervid populations has not been established for the non-hunting public (Vaughan Branch et al., 2022; Organ et al., 2012). The likelihood of CWD introduction and spread varies by locale and over time, and the severity of outcomes varies by locale, time, and species compositions on the landscape. In addition to impacts to consumptive (hunting) values, conservation and cultural values at risk can be expected to comprise of changes in non-market values that depend on the abundance and the health of free-ranging cervid populations. This study committee has been unable to identify related literature or reports that quantify the effects of CWD on conservation or cultural values, which may represent a gap in knowledge.

Hunting may occur on private or public lands, with or without access fees, and possibly through a hunting outfitter service. This variability makes it difficult to quantify the effects of CWD on the economic value of hunting free-ranging cervids. In addition, hunters may or may not respond directly through behavior change to the risk of CWD, and hunters may or may not respond to policies made in response to the risk of CWD. All these responses may vary in intensity over time as the prevalence of CWD varies and people become more familiar with the disease (Pattison-Williams et.al., 2020). The typical approach to determine the change in surplus (also known as the net benefit) to hunters is to quantify changes in the willingness to pay for hunting (which measure the benefits of hunting), net of changes in hunting related expenditures (license fees, access payments, equipment, and travel costs) and the opportunity cost of leisure with and without the effects of CWD.

While no national-level estimates of CWD-related loss of hunter welfare or expenditures exist in the current literature, there are a few state-level analyses. For example, following the discovery of CWD in Wisconsin in 2001, losses in hunter expenditures were estimated to be $55 million in 2002 and $33 million in 2003, and the reduction in household incomes due to the expenditure losses of $41mllion in 2002 ($71.2 million in 2024 dollars) and $25 million in 2003 ($42.4 million in 2024 dollars) (Bishop, 2004). That same study concluded that the total loss to Wisconsin deer hunters based on the surplus hunters generate from hunting was between $53 million and $79 million in 2002 ($92-$137 million in 2024 dollars), and $45 million to $72 million in 2003 ($76.3-$122.1 million in 2024 dollars). In a follow up study of the effect of CWD on hunting in Wisconsin, Erickson et al. (2019) found a total reduction in hunter surplus for the period of 2002-2015 of $96 million (in 2012 dollars, or $130.5 million in 2024 dollars) (Erickson et al., 2019). Given the findings of Bishop (2004) for Wisconsin, Menard et al. (2004) predicted the potential short-run effect of CWD discovery in Tennessee on Tennessee's economy. Following Bishop (2004) a 15 percent reduction in hunter days was modeled and was found to result in direct losses of $46 million (in 2004 dollars, or $76.6 million in 2024 dollars) and a reduction of almost 900 jobs (Menard et al., 2004). Considering indirect and induced effects across the economy in

Tennessee, losses were estimated to climb to $98 million (in 2004 dollars, or $163.1 million in 2024 dollars) and almost 1500 jobs lost. Losses to hunters were not estimated.

Costs and Implications of CWD for Government Agencies

Recent governmental agency costs of surveillance, testing, and management for the United States have been calculated by Chiavacci (2022) who differentiates the costs by those born by natural resource agencies and those born by agriculture/animal health agencies for 2020. Chiavacci estimated that federal government agencies collectively spent over $284.1 million on CWD-related activities between 2000 and 2021 with the USDA's Animal and Plant Health Inspection Service (APHIS) spending $203.6 million of this total (Chiavacci, 2022). State natural resources agencies and state agriculture/animal health agencies are estimated to have spent over $25.5 and $2.9 million, respectively, just in fiscal year 2020 (Chiavacci, 2022). Cost data by state from Chiavacci (2022) are provided in Appendix F. As stated previously, license and permit fees as well as excise taxes paid by hunters, anglers, boaters, and recreational shooters provide over half the funds expended by public fish and wildlife management agencies for all their conservation activities (Thompson and Mason 2022, Casellas Connors and Rea 2022; Association of Fish & Wildlife Agencies and the Arizona Game and Fish Department, 2017). These state and tribal agencies also typically bear the burden of costs from CWD related programs. Often, these costs must be reallocated from other wildlife conservation or management efforts (Chiavacci, 2022) and if the threat of CWD lowers hunter participation and license revenues, already sparse budgets for conservation can be spread even thinner.

Where CWD prevalence has been low, hunter participation and license applications have been relatively unaffected over time in many cases, although there have been instances of greater consequences when prevalence rates have risen to higher levels (Pattison-Williams et al 2020, Erickson et al 2019; Haus et al., 2017). For example, following the discovery of CWD in Wisconsin, data indicated an initial CWD-related reduction in the demand for hunting licenses of greater than five percent (Bishop, 2004). Over the years following discovery, the magnitude of this reduction was found to have diminished gradually with time (Erickson et al., 2019). The reduction in hunting permits during 2002-2015 led to a decline in permit revenues of $17 million (in 2012 dollars, or $23.1 million in 2024 dollars) (Erickson et al., 2019). Box 7.1 includes a description of hunter response to CWD in New York.

The expenses associated with CWD management represent economic vulnerabilities of agencies and underlie the importance of prevention and early detection and response capabilities—especially in states that have not had yet discovered CWD cases. State agencies may be unequipped or underequipped to meet the current challenges of CWD. Their present levels of resources and capacity may not be sufficient to address the challenges of CWD if the disease continues to spread and more cases occur.

Better Understanding of Economic Dimensions

An incomplete understanding of the economics of CWD is a detriment to an in-depth understanding of disease and its control. Although pertinent data exists on some state and federal costs and expenditures, it is not currently possible to quantify the full economic burden or economic impact of CWD on most states at any jurisdictional level and certainly not on a national scale. The inability for economists to generate economic impacts analyses has negative consequences including the inability to calculate cost-benefit analyses on CWD prevention, reduction, and control programs. Without accurate and up to date economic information, interested and affected parties may not perceive a sense of urgency to act, and decision makers and policymakers lack critical data for planning and implementing cervid programs.

CULTURAL DIMENSIONS: IMPACTS ON NATIVE AMERICANS

There are 574 federally recognized tribes in the United States, each a sovereign nation, as well as state-recognized tribes and Native American communities. It was not possible for this committee to glean the many different perspectives of these individual tribes to ascertain the threats and concerns of tribal communities related to CWD. Thus, the committee leaned on the limited published studies and the expert opinions of those identifying as a tribal member, employees of tribal natural resource agencies, or those working directly with tribal communities on CWD to develop this report. CWD may impact the economic, food security, cultural, and spiritual connection tribal nations have with cervids (Parlee et al., 2021; Maraud and Roturier, 2021; News Release[1]). Research on the impacts of CWD on tribal nations and their communities is underway in the Midwest, however more is needed to fully understand the breadth of those impacts across tribal communities more broadly.

For many Native American people living in the United States, cervids are historically important and present-day subsistence species. The negative impacts of CWD on Native Americans are not unlike those of other local communities; however, long-standing cultural practices and knowledge related to cervids bring unique cultural perspectives and concerns (e.g., Priadka et al. 2022; Moore et al. 2024). Many rely on cervid meat as a locally sourced protein source in their diets (e.g., Parlee et al. 2021; Moore et al. 2024). Native Americans may use cervids for other purposes, including the brains for tanning and hides for drums, and may derive additional benefits including shelter and community connections (Great Lakes Inter-Tribal Council Snap-Ed Program, 2015). Some tribes benefit from trophy elk and deer hunts that bring in substantial economic support (Francisco, 2023). In addition to considering how the presence of CWD might impact these practices, the surveillance and management of CWD might also infringe on sacred cultural practices that may or may not be readily changed or adapted (Francisco, 2023). Currently, any direct cultural and economic effects of CWD might be felt by those who harvest cervids on tribal lands with**,** or adjacent to areas where CWD has been detected.[2] However, it is important to recognize that tribal nations balance the perceived risks of CWD as related to impacts on economics and cultural practices while managing it as sovereign nations negotiating sometimes complicated relationships with governing bodies and regulators operating under a colonial framework (Donatuto, Satterfield, and Gregory, 2011; Donoghue, 2011; Schwabenlander et al., 2022).

Where tribal communities have not yet felt the direct impacts of CWD, tribal natural resource agencies, recognizing the need for surveillance and management in their role to protect this important cultural and food source, are already experiencing impacts on agency funding and resources (Schwabenlander et al., 2022; M. Schrage, Personal Communication, November 29, 2023). The capacity of tribal wildlife management programs is commonly limited to a single biologist with few support staff, where total natural resource agency personnel might include 1-200 employees (Thorstenson 2023; Schwabenlander et al., 2022; Schrage, Personal Communication, November 29, 2023). Tribal agencies rely on a combination of game license revenue and federal contract and grant funding for much of their management (Thorstenson 2023). Tribal natural resource agencies do not receive support through the Pittman-Robertson or Dingell-Johnson Funds (Thorstenson 2023; Casellas Connors and Rea, 2022), even though tribal members pay into these funds when they purchase fishing gear, firearms and ammunition, and other hunting supplies (Thorstenson 2023). Further, the "user pays" model of conservation funding (i.e. license, tag, permit fees) is not culturally acceptable to some tribes given hunting is viewed as a treaty right and harvests are primarily for subsistence purposes (Schrage, Personal Communication, November 29, 2023). Where state agencies do offer tribal agencies some logistical and technical support, priorities of the state do not always align with those of Tribes.

[1] See https://twin-cities.umn.edu/news-events/innovative-collaboration-between-tribal-communities-and-u-m-slow-cwd-spread#:~:text=As%20of%20June%202023%2C%20CWD,Red%20Lake%20Band%20of%20Chippewa (accessed June 2015).
[2] See https://www.usgs.gov/media/images/distribution-cwd-relation-tribal-lands-us (accessed November 5, 2024).

Box 6.3 describes a midwestern tribal collaborative effort to coordinate CWD surveillance and management. However, tribal wildlife programs often lack the staff and time to apply for and administer grants such as the USDA-APHIS cooperative agreements,[3] and a competitive process is not conducive to building sustainable, long-term surveillance programs (Moore, communication with the committee, December 14, 2023; Schrage, Personal Communication, November 29, 2023). A new law through the Chronic Wasting Disease Research and Management Act (H.R.5608 - 117th U.S. Congress) governs the distribution of funds to support the research and management of CWD and specifies that USDA will enter into cooperative agreements with state and tribal agencies to administer the funding program. Continuation of the current competitive and burdensome funding process to distribute CWD support to tribal agencies will not serve the needs of those agencies. In contrast, the Indian Self Determination and Education Assistance Act (U.S. Department of the Interior, 1996) provides a mechanism for the Department of the Interior and Department of Health and Human Services to contract with tribes such that tribes have the necessary support "to operate programs serving their tribal members and other eligible persons." Over time, a series of amendments were created to "increase tribal participation in the management of Federal Indian programs and to help ensure long-term financial stability for Tribally-run programs" and to "remove many of the administrative and practical barriers" to accessing funding. Under the direction of Congress, the two federal agencies worked in cooperation with tribes and tribal organizations to co-develop regulations for implementation (U.S. Department of the Interior, 1996). This is an example of a model for transferring funds to tribes. There is need for similar models that make CWD funding more accessible and sustainable for tribal-led CWD management (Moore, personal communication, November 28, 2023). As the impact of CWD on cervid harvest and use is a food sovereignty issue, the USDA's commitment to consultation, better support, and partnership with tribal nations in empowering their food sovereignty right is promising for resolving present funding challenges. Supporting tribal priorities and capacity-building for CWD management by tribal nations may open doors to better coordination in and improved control of CWD through co-stewardship of cervid populations (e.g., Moore et al., 2024).

[3] See https://www.aphis.usda.gov/sites/default/files/fy2024-tribal-wild-cervid-cwd-funding-opportunity-announcement.pdf.; see https://www.usda.gov/media/press-releases/2023/12/06/white-house-tribal-nations-summit-usda-fulfills-long-standing.

8
Future Considerations

This report describes how new knowledge about chronic wasting disease (CWD) continues to be generated through laboratory research, field studies, and management experience. The committee's statement of task (see Box 1.2) directs the committee to draw conclusions about the state of knowledge with respect to CWD. As such, this report is a retrospective analysis of research, understanding, and practices to date, and the conclusions presented herein are based on that knowledge to date. A large body of research and knowledge about CWD has been generated in previous decades. Nonetheless, knowledge about specific CWD-related topics may be incomplete, may not be broadly accessible, or may be based on preliminary or unpublished research or data. As a result, the state of knowledge in those topical areas is often less mature or inconsistently shared or applied among decision makers.

CWD is a dynamic and complex emerging infectious disease, the control of which differs somewhat from other diseases that have affected free-ranging or captive cervids in the United States. Inconsistent surveillance across the United States makes it impossible to accurately determine the extent of true geographic distribution of CWD; however, ample evidence suggests the number of cases and geographic spread of the disease have increased over time. As the disease expands across North America, opportunities to manage the disease at local or regional levels becomes increasingly challenging, and new management strategies will be necessary. This final chapter synthesizes the report's main conclusions regarding the implications of CWD transmission, spread, and the effectiveness of control activities to shift the disease trajectory toward positive outcomes. It may still be possible to modify or adopt practices to help slow transmission in many areas; however, a new commitment to sustained disease management efforts, a sense of urgency, and call to collaborative and consistent action are necessary.

Gaps in scientific knowledge regarding different aspects of CWD challenge the adequate long-term control of the disease. Box 8.1 provides examples of such knowledge gaps. More coordinated research will be necessary to fill some of those gaps, but it is possible that many questions could be answered with using existing but inaccessible information. Frustrating for the committee was its inability to come to certain conclusions because Federal and state agencies were unable to share certain kinds of information with the committee. The importance of information and data access has long been recognized as foundational to CWD management. Federal, tribal, and state agencies formed tasked forces to develop action plans for communication, scientific technical information dissemination, and data storage (e.g., Chronic Wasting Disease Task Force, 2002), but those data are still not largely available in useful and accessible formats. Much knowledge held about the disease is still implicit (i.e., in quadrant III in the knowledge matrix presented in Box 1.3), often based on undocumented or inaccessible research and surveillance data, and shared only through, for example, discussions held in meetings following the Chatham House Rule.[1]

A concern of the committee is the extent to which research and management decisions are impacted by the lack of access to existing information. Decisions made with incomplete information could be fraught with uncertainty that can affect the strategic efficacy and cost-effectiveness of management decisions. The lack of information and data accessibility seems to be indicative of a larger problem—a lack of collaboration among critical interest groups. Addressing CWD is made even more challenging by significant differences in interests, values, beliefs, and behaviors resulting in a lack of a

[1] The Chatham House Rule states, "When a meeting, or part thereof, is held under the Chatham House Rule, Participants are free to use the information received, but neither the identity nor the affiliation of the speaker(s), nor that of any other participant may be revealed." See https://www.chathamhouse.org/about-us/chatham-house-rule (accessed June 22, 2024).

unified purpose and a lack of common goals and outcomes. Even when agreement on reducing or controlling the disease is demonstrated, acceptable strategies are dependent on individuals' willingness to act and change behavior. Evidence-based information and knowledge need to be the foundation for determining the critical actions and programs that can prevent or reduce the transmission and spread of CWD. The inability to share information and data limits the general state of knowledge regarding CWD and is detrimental to further understanding and control of the disease.

The conclusions in this chapter are intended to inform the design of a strong action-oriented and integrated strategy to reduce the transmission and further geographic spread of the disease. They are organized in the order addressed in the report and their order does not represent prioritization.

BOX 8.1
Information Needs

Understanding the transmission and spread of CWD and the efficacy of CWD management strategies could be improved through information gathering and analyses. The following list synthesizes some of the information needs identified by the study committee. The list is neither exclusive nor prioritized.

- A comprehensive compilation and analysis of previous epidemiological investigations of CWD outbreaks in captive cervid facilities, with an emphasis on occurrences in HCP populations
- A retrospective assessment and comparison of local and large-scale disease management and control activities and their outcomes
- More information on CWD strain discrimination, characterization, and standardization including observed impacts of different strains on pathogenesis and disease susceptibility across cervid species and potential spillover to humans and non-cervid species
- An evaluation and determination of the linkages between environmental prion sources and infectious dose for greater understanding of how environmental sources may contribute to CWD transmission and spread in free-ranging and captive cervid populations
- Data regarding improvements and validation of diagnostics for control and management interventions
- Corroborated scientific evidence to expand relevant knowledge on the potential role of host genetic variability on the dynamics of CWD infection and disease control in captive cervid herds
- Improved understanding of how human dimensions (e.g., behaviors across interest groups) positively and negatively impact management practices

DRIVERS OF TRANSMISSION

Conclusion 1: Multiple drivers and epidemiological factors affect CWD transmission and infectivity. The precise roles, interrelationships, and relative importance of these factors are not fully understood, cannot be fully quantified, and may differ for captive and free-ranging cervid populations.

The multiple drivers and factors affecting transmission and geographic spread of CWD, their relationships, roles, and relevance are not completely understood. Studying CWD in natural systems is difficult and detailed understanding of many aspects of this disease derives from controlled studies conducted in either a limited number of natural or experimental hosts or *in vitro*. However, data derived from artificial laboratory approaches may have limited application to analogous processes occurring naturally in the environment, including transmission. For example, the infectious dose, effects of repeated exposure, CWD prion persistence in the environment, duration of incubation and host shedding of the pathogen, the molecular makeup of the prion itself, and host genetic differences influence the likelihood of transmission in a laboratory setting but their importance have not been quantified in natural settings.

Furthermore, there are numerous anthropogenic factors that likely impact CWD transmission and geographic spread, but definitive, evidence-based information regarding those is lacking.

TRANSMISSION POTENTIAL TO HUMANS AND OTHER SPECIES

Conclusion 2: As of this writing, no cases of CWD transmission to humans have been diagnosed or reported, nor has natural transmission to non-cervid animal species been detected.

As the prevalence and geographic distribution of CWD increases, so does the risk of exposure to other potentially susceptible species, and the risk for generation of new CWD strains. Current evidence suggests that the transmission of CWD to non-cervid species is unlikely in natural scenarios, with no cases of CWD transmission to humans or other animal species having been reported. However, experimental evidence and the existence of multiple CWD strains with unknown properties does not preclude the possibility of transmission to non-cervid species. Epidemiological and experimental evidence strongly suggest that humans are resistant to CWD prion infectivity, but this barrier is not absolute and the zoonotic potential of each unique CWD strain needs to be carefully assessed. Evidence from studies incorporating experimental amplification assays and surrogate models of human susceptibility (including some—but not all—species of non-human primates and varieties of transgenic mice expressing the human prion protein) suggests that certain strains of CWD prions may have some potential to induce the human prion protein to misfold and cause disease (Kong, et al., 2005; Sandberg et al., 2010; Wang et al., 2021; Race et al., 2018; Race et al., 2009). However, those data need to be interpreted with caution, as low levels of prions, perhaps below the level of clinical relevance, may be amplified, while route of inoculation (e.g., oral versus direct injection into the brain) and strain differences may account for some of the observed variability. Experimentally, CWD prions can be made to infect multiple species that inhabit the same geographic region as cervids (e.g., pigs, raccoons, cattle, and rodents). That said, most of these transmissions were achieved by intra-cerebral inoculations (i.e., injection directly into the brain) and not natural routes of exposure. CWD prions can occur in fecal material of free-ranging carnivores such as cougars, coyotes, and crows, but the prions pass through their digestive systems without necessarily infecting them (Nichols et al., 2015; Baune et al., 2021; Inzalaco et al., 2024). Whether infection can be achieved in non-cervid species by more natural routes of exposure (e.g., ingestion) is not known but the possibility cannot be discounted.

SPREAD OF CWD

Conclusion 3: The known geographic distribution of CWD is expanding. However, the distribution is incompletely understood and likely underestimated. Inconsistent surveillance has compromised knowledge about changes in CWD distribution over time in the United States.

At the time of this writing, CWD has been reported in 35 states in the United States. However, the timeline(s) for its geographic spread cannot be inferred reliably based only on the chronology of first detections in a region. Surveillance and monitoring present unique challenges among captive and free-ranging herds. Organized surveillance was virtually absent in the United States prior to 1997, and records from subsequent surveillance, outbreak investigations, reporting, and documentation of human-assisted translocation of cervids or cervid parts is incomplete. CWD surveillance in both captive and free-ranging cervids has improved generally since 2002, although the data and details of surveillance efforts were largely unavailable to this study committee and are, apparently, not readily available to either the broader scientific community or the public. This lack of transparency impedes both the general understanding of CWD, perspectives about historical and current extent of CWD, the effectiveness of surveillance activities, public trust in agency management, and ultimately, the control of CWD in both free-ranging and captive cervids. These impediments are compounded by the fact that current surveillance practices

and participation remain inconsistently applied on a national scale and thus, CWD distribution is likely underestimated.

Conclusion 4: Natural movement of infected cervids and other epidemiological factors are responsible for the local distribution of CWD. Human-mediated movement of infected cervids (i.e., transport of live, dead, captive, or free-ranging cervids) and infected cervid products for commerce, recreation, conservation, and other purposes increases the likelihood of CWD spreading to new geographic areas in unpredictable ways.

Outbreaks of CWD rely on the introduction of infectious prions to new localities. The spread to these localities is often facilitated by moving infectious hosts, either live cervids or cervid carcasses, and potentially by moving other cervid products containing CWD prions (e.g., antler velvet and urine), across long distances. The distances between known and some 'new' foci are difficult to account for based solely on observed natural cervid movements. Free-ranging cervids may move extensively across their home ranges, causing the disease to radiate from its point of introduction. The commercial transport of captive cervids has unwittingly moved the disease across state and even international boundaries. The result of undocumented anthropogenic movement of CWD is less predictable than natural mechanisms for spreading the disease. While numerous bans have been implemented prohibiting the movement of select portions of hunter-harvested cervids across jurisdictions through state and provincial carcass import regulations,[2] compliance with these regulations is not well studied. Given the risk of environmental transmission and the role of CWD-infected cervids in environmental contamination, efforts to reduce the movement of infectious cervids or cervid parts will reduce the risk of direct or indirect transmission to new hosts or landscapes. Once a new locality has been contaminated with CWD, human behavior at local scales (e.g., improper disposal of carcass parts; use of attractants or bait to encourage aggregation of cervids; handling of CWD-positive cervids) may increase the spread of the disease. Further, the natural movement ecology of cervid populations may foster increased direct and indirect transmission risk among deer family groups and those using spatially overlapping environments.

DIAGNOSTIC METHODS

Conclusion 5: Official USDA postmortem CWD diagnostic approaches are useful for disease surveillance in both free ranging and captive cervids. Newer, as yet unapproved, detection approaches may have more wide-ranging applications, including live-animal testing and screening of cervid byproducts, environmental surfaces, and other relevant materials.

Since the early 2000s, CWD detection and surveillance has largely relied on standardized immunohistochemistry (IHC) or enzyme-linked immunosorbent assay (ELISA) protocols for the detection of the misfolded prion protein in lymphoid and neural tissues – predominantly in tissues collected postmortem. These approaches have been used by state, federal, and tribal agencies to identify newly infected free-ranging and captive cervid herds and estimate disease prevalence either passively during routine sampling or actively during depopulation, for example. These methods, although accurate, have not been widely applied for live animal testing and cannot be used in surveilling environmental or biological components (e.g., soil, plants, insects, or bodily waste) that may act as sources of CWD transmission.

At roughly the same time that these "conventional" approaches were first approved for widescale CWD testing across North America, development of amplification-based tests for prion disorders was underway, initially through the protein misfolding cyclic amplification (PMCA) assay and eventually the real time quaking-induced conversion (RT-QuIC) assay. These amplification assays have demonstrated arguably higher levels of sensitivity than their conventional counterparts, allowing use of samples

[2] See https://cwd-info.org/state-and-province-carcass-import-regulations/ (accessed June 22, 2024).

collected from live cervids, and have proven useful in evaluating a range of matrices including bodily fluids and environmental samples that are untestable by IHC and EIA. Despite their potentially higher levels of sensitivity and utility in assessing non-traditional samples, these amplification assays require further validation and inter-laboratory standardization and have not yet been approved by regulating bodies.

New knowledge and improved diagnostic and detection methods to accurately surveil potential environmental contamination and to monitor changes and emergence of new strains are especially needed to confront challenges where the disease has become established.

CONTROL

Conclusion 6: Well-founded epidemiological principles inform strategies for CWD prevention or control in both captive and free-ranging cervids, beginning with effective early ongoing surveillance and followed by timely aggressive sustained local response upon the presence of CWD being discovered. Although imperfect, methods based on those principles can reduce or prevent large increases in prevalence and slow the spread of CWD when properly applied.

Although CWD has some unique characteristics regarding transmission and spread, it, nevertheless, follows the same epidemiological principles as other contagious infection diseases. The traditional epidemiologic triad model holds that infectious diseases result from the interactions among an agent, host, and environment. Transmission occurs when the agent (prion) leaves the host/reservoir (CWD-infected cervids) through a portal of exit and is conveyed by some mode of transmission (direct or indirect) and enters through a portal of entry (oral, etc.) to infect a susceptible host. This sequence is referred to as the chain of infection. The components of the chain regarding CWD have been described throughout this report. All infectious diseases whether the agent is a virus, bacterium, fungus, parasite, or prion or whether the disease involves humans or animals, can be described and explained by this foundational principle. (Ref. CDC, Principles of Epidemiology, 2nd ed., Atlanta, HHS, 1992).

Knowledge of the portal of exit and entry and the mode of transmission provide a basis for determining appropriate control measures. In general, control measures are usually directed against the segment of the infection chain that is most susceptible to intervention. For other TSEs, for example, the BSE epidemic was mostly limited and stopped by breaking the transmission cycle by removing BSE prions from animal feed thus eliminating the portal of entry to a new host. For scrapie the control strategy focuses on controlling environmental contamination but especially by strengthening the resistance of a new host, via genetic selection, and breaking the cycle of new infections.

Box 6.4 lists potential methods and strategies being used to help control CWD. All the tools are based on ways to break the chain of infection and interrupt transmission and spread of the disease. The tools can be used singularly or in combination and are often used based on available resources, levels of endemicity, farmed versus free ranging populations, host variability, environmental issues, etc. Understanding and implementing the use of these tools can be improved through further studies and experiences, however, the basic epidemiologic principle, cycle of infection, is still the time-proven foundation that can guide interventions. The state of knowledge on many of these factors can be expanded to improve and inform new strategies; however, the proper and sustained use of these tools and understanding how they might break the infection chain allows us to use current methods, that are still valid and available, to control and prevent CWD as new strategies emerge and are validated.

As reflected in this report, there is sufficient knowledge to help slow the spread and reduce transmission of CWD while new scientific knowledge, advances, and evidence-based control strategies are developed. Current understanding, albeit incomplete, is sufficient to inform comprehensive control strategies and for prioritizing needs for further investigation. For example, the existing knowledge that infected host cervids, their residual secretions and excretions, and their carcasses are sources of infectivity can inform measures for CWD control. Such control measures include risk-based and targeted culling and increased hunting pressure on infected herds to control prevalence, baiting and feeding restrictions to reduce cervid aggregation, appropriate carcass handling, and compulsory surveillance and commercial

movement regulations to curtail spread (see Box 6.4 and Chapters 4 and 6). In contrast, the potential contributions of non-host sources of CWD prions (e.g., contaminated feed) to transmission and geographic spread are not as well understood and have yet to be considered fully in the context of control. Thorough epidemiologic analyses of new infections are warranted to improve understanding of risk factors and improve disease prevention and biosecurity measures. Collaborative efforts to understand the collective portfolio of evidence and to convert existing but inaccessible or anecdotal information into accessible and actionable information will enhance the collective ability to blunt the effects of the disease in the short-term while research continues to develop longer-term control solutions.

Conclusion 7: Differing philosophies and approaches to CWD management adopted by agricultural and wildlife management authorities at different levels of government impact the effective control of CWD in the United States.

The diverse viewpoints of interested parties and the differences in authorities in local, state, tribal, and federal jurisdictions complicate efforts to control CWD. The epidemiological characteristics of CWD, the uncertainties regarding its transmission and spread, the long lead times before animal mortality is observed, and a lack of demonstrated human harm from the disease makes crafting and justifying control policies in response to CWD challenging. Furthermore, CWD is managed by local, state, tribal and federal officials which operate under different authorities, jurisdictions, regulations, guidelines, social pressures, and management strategies, often with inadequate resources. The result has been a patchwork of prevention and control strategies that are unevenly and inconsistently adopted, implemented, and evaluated.

Management objectives of responsible agencies differ, and therefore the approaches to controlling CWD are likely to remain, different. State and federal agricultural agencies are largely responsible for managing captive cervids (generally considered livestock), primarily for the economic benefit of producers and for meeting market demand for cervids and their products. The goals of state, tribal and federal wildlife management agencies often focus on maximizing recreational, economic, and societal values associated with free-ranging cervids. For example, reducing deer populations at a local or regional level to limit transmission can directly conflict with hunter and wildlife enthusiast preferences for seeing greater numbers of cervids on the landscape. Tribal agencies may have the additional challenges of balancing cultural and traditional values, food sovereignty, and a subsistence economy with wildlife management priorities, while also having limited agency capacity and high administrative burden in acquiring federal funding and grant management.

Given current strategies and management practices, it cannot be expected that the disease can be eradicated in areas where it is already well-established, but prevalence could be controlled and spread can be slowed even as additional control tools can be developed, validated, and implemented. The worst-case scenario is that the disease will spread across the entirety of the country, wherever cervids are present. A better understanding of the underlying genetics of disease resistance, coordinated and complementary control strategies, and the development of and access to diagnostic approaches are important considerations for overcoming the present impediments to CWD control. In addition, it will take honest, concerted, collaborative, patient, dedicated, and consistent long-term effort from numerous agencies and interested and affected parties to identify and weigh conflicting scientific, social, and economic priorities, and come to agreement on appropriate solutions for protecting both captive and free-ranging populations. Greatest success is likely when prevention and management plans are introduced before disease is detected, providing opportunity to slow the spread when the prevalence is low. Supporting further research into new effective interventions and better utilization and implementation of known control strategies would inform future adaptive management decisions for free-ranging populations.

Conclusion 8: Prevention is key to controlling the spread of CWD given that existing tools and technologies make eradication of CWD in captive or free-ranging cervid populations, once

established, improbable. Ongoing and effective surveillance programs can facilitate early detection and response.

Managing CWD is complex because (a) surveillance in free-ranging cervids is constrained; (b) environmental contamination and its contribution to indirect transmission are persistent; (c) early diagnosis of infection in live cervids is challenging; (d) the lack of vaccines against or therapeutics to treat CWD; and (e) the difficulty recruiting interested and affected parties to participate in control. Once CWD is established in a free-ranging cervid population, CWD is unlikely to be eradicated with existing scientific methodologies, technologies, and current management and surveillance practices. However, there are limited examples where early detection of CWD and prompt, aggressive response resulted in the local apparent eradication of the disease and the prevention of spread to nearby areas (see Chapter 6 and Box 7.3). Ongoing and effective surveillance programs allow early detection of CWD and sustained adaptive management to respond to changing conditions. Strategies that prevent the introduction of CWD to a region or captive facility are important in controlling further spread of the disease, but ongoing surveillance once controlled in a location is crucial to detect disease re-emergence.

Once CWD has become established in an area, successful management and control strategies may keep CWD prevalence at low levels. Maintaining low CWD prevalence may limit the buildup and overall contribution of environmental contamination to ongoing transmission, but it is yet unclear how long low prevalence levels can be suppressed with active management. Environmental contamination is not homogeneous across the landscape; the persistence of CWD prions is dependent on soil type, and potentially other factors such as weather, vegetation, heterogeneities in the landscape, and sympatric animal species. This complicates the development and implementation of environmental contamination mitigation strategies in captive cervid settings or specific habitat manipulations for free-ranging cervids. Presently, there are no feasible environmental treatments or habitat modifications that effectively limit or eliminate prions.

Quickly identifying CWD-infected captive herds and removing them from the landscape are likely important in controlling disease spread. Infection of captive cervid herds can be limited with strong biosecurity and preventive management; however, these cervids are still at risk, particularly in areas where the disease occurs among free-ranging cervids. It is reasonable to expect that the USDA CWD HCP has contributed to limiting the interstate spread of CWD but, in its current form, the program may lack the scope, resources, and jurisdictional authority to fully mitigate the risks of CWD in captive cervids. Hence, an in-depth epidemiological analysis of CWD-infected herds, including those enrolled and unenrolled in the HCP, would be beneficial to better understand key risk factors, program reductions to CWD risk, and necessary improvements in disease prevention and biosecurity.

Conclusion 9: Genetic selection, vaccines, environmental decontamination, and therapeutic options are being investigated as tools for CWD control but need further inquiry and review. Although none of these approaches can, at present, replace existing forms of management and control, in the future they may, in combination with current methods, reduce CWD on the landscape.

Genetic resistance, vaccines, and therapeutic options have garnered hope among some as solutions for CWD control. At this time, there is insufficient data and validation to determine their effectiveness. As discussed in Chapter 6, studies using selective breeding, incorporating both *PRNP* and genome wide associations with susceptibility, as a management tool in the control of CWD in captive white-tailed deer and elk herds are in their early stages. The impacts of different rates and lengths of exposure in these herds, as well as exposure to different CWD strains, have not been determined, which may limit utility of selective breeding for managing CWD in captive cervids, particularly those that might preferentially infect cervids with *PRNP* genotypes presently considered less susceptible to infection. The downstream effects of infected animals with extended incubation times shedding infectious prion in the environment for prolonged periods have also not been addressed. The applications for genetics in managing CWD in free-ranging populations have not been described or addressed. Longitudinal studies in

both captive and free-ranging populations would facilitate a better understanding of genetics on relative susceptibility to prion infection and prion shedding, as well as applicability in captive or free-ranging conditions.

Vaccines, environmental decontamination, and therapeutics have also been investigated. The results of past and ongoing vaccine development efforts have been inconsistent. Without better knowledge of cervid and prion biology, these strategies may not be optimally utilized. Barriers to implementation remain, especially in free-ranging cervids, are the difficulties associated with vaccine delivery, the potential uncertainties in CWD prion shedding in vaccinated cervids and protection against a range of different CWD strains. Finally, environmental decontamination protocols and therapeutic options for CWD and other livestock prion disorders are practically non-existent. The development and implementation of environmental contamination mitigation strategies in captive cervid settings or specific habitat manipulations for free-ranging cervids is challenging because heterogeneities in environmental contamination within landscapes. Although there is limited evidence for efficacy in reversing the course of protein misfolding or reducing infectivity in biological samples, there is no evidence of whether this is effective in cervids or in the environment. While each of these avenues may be important in CWD management in the future, current strategies focusing on active surveillance and regulations covering the movement of cervids and their byproducts will help slow the transmission and spread until more effective management options are developed, validated, and implemented.

SOCIAL AND JURISDICTIONAL FACTORS

Conclusion 10: Human behaviors can influence the transmission, spread, and consequences of CWD. Interest groups hold diverse viewpoints regarding the seriousness of CWD and about its spread, prevention and control; their decisions may not always be informed or influenced by the best available science.

CWD touches the lives, livelihoods, and enjoyment of many interested and affected parties. Numerous and diverse organizations, government agencies, interested and affected parties, and disciplines with differing points of view, incentives, interests, values, policies, and authorities have concerns regarding CWD. The diversity of views can make it difficult to reach consensus when interpreting information and deciding disease management strategies. The different views, cultures, relationships, levels of trust, and senses of urgency among groups and individuals are often in opposition and can impact control of the disease. The lack of access to data and information can contribute to the lack of trust, coordination, and cooperation among interested and affected parties. Chapter 5 describes some of the evidence of human actions that may contribute to the geographic spread of the CWD, and Chapter 7 describes some of the drivers for those actions. For example, the transport of infected cervids or carcasses may be responsible for disease spread. There are agencies, interest groups, and individuals that make decisions to avoid transporting potentially infected cervids and carcasses to slow the spread of CWD. However, there are also groups and individuals whose livelihoods may depend on transporting cervids, who may not have the resources to change their behaviors or actions, whose way of life may be threatened by changing rules, or who may be unaware of the CWD-related risks because of either a lack of information or because of the easy availability of misinformation (see Box 8.2). State and local jurisdictions are often more reactive to social, political, and economic forces rather than scientific evidence and such decisions and actions may thwart efforts to slow the spread of CWD.

As concluded earlier, the multiple viewpoints and differences in authorities, available resources, and disease management practices across jurisdictions addressing CWD result in inconsistent and sometimes incompatible management. Lack of mechanisms for communication and coordination around data collection and information sharing across jurisdictions precludes an evaluation of control measures and their efficacy. Without coordination among individual jurisdictions, the knowledge held by individual jurisdictions cannot be leveraged. For example, logic dictates that the USDA Herd Certification Program (HCP) may have had a positive impact on limiting the interstate spread of CWD. However, the committee

has no access to any existing data that might quantify those impacts, and CWD continues to be identified in HCP herds. Any data collected would likely be at the state or local levels, and there is little to no ability to get or share epidemiological information regarding CWD in captive HCP or non-HCP herds. The HCP provides an important framework for CWD management in captive herds that could be refined and expanded.

BOX 8.2
Knowledge to Counter Misinformation

Knowledge is the basis of effective communications and can counter misinformation. Research on CWD could be both broadened, accelerated, and made more collaborative among researchers and study teams, with an emphasis on interdisciplinary and systems approaches, sharing information, and coordinating and prioritizing work. Social scientists, decision scientists, and economists have important roles in understanding the actions of interested and affected parties, identifying their potentially conflicting priorities, and helping to create common interests and acceptable actions.

While management of CWD is necessarily local and needs to be responsive to unique local conditions, larger-scale coordination also seems necessary because CWD does not respect jurisdictional boundaries. Collaborative strategies are needed to manage the disease as well as provide the flexibility needed by officials. Scientific challenges are often compounded by social, political, and economic challenges.

Translation of scientific knowledge regarding CWD into decision-making requires making full use of knowledge and understanding of CWD gained through collaborative and coordinated open sharing across disciplines, government and private organizations, and political levels. Collaboration and coordination across multiple and differing partners are critical to mitigate the ongoing negative impacts of the disease on population levels, minimize socio-economic impacts of the disease, and launch effective, sustainable interventions and assessments of management alternatives. Sociologists, economists, behaviorists, anthropologists and others could be included in studying and understanding the impact and spread of CWD to appreciate its impact on local populations and communities.

ECONOMIC ANALYSES

Conclusion 11: Existing data gaps make CWD-related economic measurements and analyses difficult to quantify. These deficiencies can result in a lack of appreciation of the full impact of the disease and in the inability to evaluate and compare the direct and indirect costs and benefits of various management strategies.

There is sufficient evidence to indicate that CWD is a costly disease and that it is becoming more costly and consequential as it continues to spread and infect more populations. However, the economics of CWD are not well understood and this is detrimental to an in-depth understanding of the impacts of the disease and the need for its control. The inability to generate economic impacts analyses leads to the inability to calculate cost-benefit analyses on CWD prevention, reduction, and control programs. Expenses associated with CWD management represent economic vulnerabilities for the federal, tribal, state, and local entities with CWD-related authorities and responsibilities may be unequipped or underequipped to meet the current challenges of CWD, and resources currently available may be insufficient to address CWD as it continues to spread. Tribal wildlife management entities are further constrained by the lack of resources and staff and levels of administrative burden associated with the complicated processes for transfer of Federal funding and grant management.

Although some data regarding state and federal costs and expenditures are available, the full economic burden or impact of CWD on most states, cannot be quantified. Costs and benefits of CWD management options will depend on multiple variables including the current and predicted populations of both captive and free-ranging cervids, local economies, and a range of human dimensions. At present most jurisdictions can only produce rough estimates of the numbers and locations of free-ranging cervids and herds. Analyses of captive and free-ranging population levels in a wide variety of locales, and in concert with robust CWD surveillance programs, would support cost-benefit analyses of localized CWD management activities. Estimates of opportunity cost functions allow the prediction of future consequences of additional captive or free-ranging herds becoming infected with CWD. The lack of accurate and up to date economic information may result in some entities and individuals undervaluing the impacts of CWD and diminish any sense of urgency to act on CWD.

CONCLUDING THOUGHTS

It is known that CWD results in increasing mortalities in cervids, negatively affecting individual cervids and, at sufficiently high infection prevalence, their population growth rates (particularly in deer) over time. Localized impacts of CWD may differ depending on a variety of ecological and human factors. The potential social and ecological ramifications of the increasing spread of CWD are severe that could include large scale economic losses to affected states, and to the commercial efforts related to captive cervids. CWD creates a more challenging environment to rear captive herds, including direct and indirect economic losses associated with increased regulations, guidance, and policies. It also results in substantial costs in money and time for local, state, tribal, and federal government agencies.

CWD is likely to be a part of the North American landscape for the foreseeable future. Infected cervids have a long incubation period. Through much of the incubation period, including when cervids show no outward signs of the disease, prions are shed to the environment and become a persistent threat. The considerable knowledge about CWD gained in more than 40 years can be drawn upon, in conjunction with the sustained and collaborative commitment of resources and effort by all entities with an interest in CWD and cervid health, to slow the spread of the disease in the near term. A sustained collaborative effort over decades using the accumulation of the credible CWD-related scientific knowledge (e.g., what is known and unknown, what is important and not important) is the foundation for long-term effort to change the trajectory of CWD in free-ranging and captive cervids.

The nature of science in any area is that it evolves and is refined as more is learned and confirmed and validated. As such, the state of knowledge is still evolving, with healthy scientific debate occurring over investigative results that sometimes appear contradictory. And although CWD has been researched for decades, there are many investigations and data that have yet to be documented, validated, and made readily available to those trying to understand or make decisions related to CWD management. There are gaps in knowledge related to multiple aspects of CWD, its epidemiological and biological complexity, and the diversity and competing views of various interested and affected parties make CWD challenging to address and control. However, the ability to adopt and implement strategies already known and scientifically supported can result in a productive, cost-effective set of interventions capable of slowing the transmission and spread. Science, collaborative research, and experience continue to provide new knowledge and understanding of CWD in free-ranging and captive cervids. Further exploration of how to apply this knowledge, particularly to free-ranging populations, would improve management decisions aimed at altering the trajectory of CWD in the US and perhaps beyond.

References

21 U.S. Code § 623 – Exemptions from Inspection Requirements. https://www.law.cornell.edu/uscode/text/21/623 (accessed November 11, 2024).

Abdelaziz, D.H., S. Thapa, B. Abdulrahman, L. Lu, S. Jain, and H.M. Schatzl. 2017. Immunization of cervidized transgenic mice with multimeric deer prion protein induces self-antibodies that antagonize chronic wasting disease infectivity in vitro. *Scientific Reports* 7(1):10538. https://doi.org/10.1038/s41598-017-11235-8.

Abdelaziz, D.H., S. Thapa, J. Brandon, J. Maybee, L. Vankuppeveld, R. McCorkell, and H.M. Schätzl. 2018. Recombinant prion protein vaccination of transgenic elk PrP mice and reindeer overcomes self-tolerance and protects mice against chronic wasting disease. *The Journal of Biological Chemistry* 293 (51): 19812-19822. https://doi.org/10.1074/jbc.RA118.004810.

Ableman, A., K. Hynes, K. Schuler, and A. Martin. 2019. Partnering with Taxidermists for Improved Chronic Wasting Disease Surveillance. *Animals* 9(12):1113. https://doi.org/10.3390/ani9121113.

Abrams, J.Y., R.A. Maddox, A.R. Harvey, L.B. Schonberger, and E.D. Belay. 2011. Travel history, hunting, and venison consumption related to prion disease exposure, 2006-2007 FoodNet Population Survey. *Journal of the American Dietetic Association* 111(6):858-63. https://doi.org/10.1016/j.jada.2011.03.015.

Acemoglu, D., D. Laibson, and J.A. List. 2019. Microeconomics. Pearson Education Limited.

Adkin, A., V. Webster, M.E. Arnold, G.A.H. Wells, and D. Matthews. 2010. Estimating the impact on the food chain of changing bovine spongiform encephalopathy (BSE) control measures: The BSE Control Model. *Preventive Veterinary Medicine* 93(2):170-182.

Ågren, E.O., K. Sörén, D. Gavier-Widén, S.L. Benestad, L. Tran, K. Wall, G. Averhed, N. Doose, J. Våge, and M. Nöremark. 2021. First Detection of Chronic Wasting Disease in Moose (Alces alces) in Sweden. *Journal of Wildlife Diseases*. 57(2):461-463. https://doi.org/10.7589/jwd-d-20-00141.

Almberg, E.S., P.C. Cross, C.J. Johnson, D.M. Heisey, and B.J. Richards. 2011. Modeling Routes of Chronic Wasting Disease Transmission: Environmental Prion Persistence Promotes Deer Population Decline and Extinction. *PLOS ONE* 6(5):e19896, https://doi.org/10.1371/journal.pone.0019896.

Anderson, A. and W.H. Chomphosy. 2014. The impact of chronic wasting disease on the geographic distribution of the US captive cervid industry. Western Economics Forum 13:11-20.

Anderson, D.P., B.J. Frosch, and J.L. Outlaw. 2007a. Economic Impact of the United States Cervid Farming Industry. Texas A&M University (Agricultural & Food Policy Center, Department of Agricultural Economics, Texas Agricultural Experiment Station, Texas Cooperative Extension, Texas A&M University, Research Report 07-4). https://afpc.tamu.edu/research/publications/480/rr-2007-04.pdf.

Anderson, D.P., B.J. Frosch, and J.L. Outlaw. 2007b. Economic impact of the Texas deer breeding industry. Texas A&M University (Texas A&M University, Agricultural & Food Policy Center).

Anderson, C. 2023. Characterizing U.S. Agency Approaches to Cervid Carcass Disposal in the Context of Chronic Wasting Disease Management: A Multi-State, Mixed-Methods Analysis. University of Minnesota.

Anderson, S., K. Leong, K. Musgrave, J. Powers, and D. Wong. 2010. Zoonotic Disease Risk Perception and Use of Personal Protective Measures among Wildlife Biologists: An Application of the Health Belief Model. *Human Dimensions of Wildlife* 15(3):221-228. https://doi.org/10.1080/10871200903460252.

Andréoletti, O., P. Berthon, D. Marc, P. Sarradin, J. Grosclaude, L. van Keulen, F. Schelcher, J.M. Elsen, and F. Lantier. 2000. Early accumulation of PrP(Sc) in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie. *Journal of General Virology* 81(Pt 12): 3115-3126. https://doi.org/10.1099/0022-1317-81-12-3115.

Angers, R.C., H.E. Kang, D. Napier, S. Browning, T. Seward, C. Mathiason, A. Balachandran, D. McKenzie, J. Castilla, C. Soto, J. Jewell, C. Graham, E.A. Hoover, and G.C. Telling. 2010. Prion strain mutation determined by prion protein conformational compatibility and primary structure. *Science* 328(5982): 1154-8. https://doi.org/10.1126/science.1187107.

Angers, R.C., S.R. Browning, T.S. Seward, C.J. Sigurdson, M.W. Miller, E.A. Hoover, and G.C. Telling. 2006. Prions in skeletal muscles of deer with chronic wasting disease. *Science* 311(5764):1117. https://doi.org/10.1126/science.1122864.

Angers, R.C., T.S. Seward, D.Napier, M. Green, E. Hoover, T. Spraker, K. O'Rourke, A. Balachandran, and G.C. Telling. 2009. Chronic Wasting Disease Prions in Elk Antler Velvet. *Emerging Infectious Diseases* 15.

Argue, C.K., C. Ribble, V.W. Lees, J. McLane, and A. Balachandran. 2007. Epidemiology of an outbreak of chronic wasting disease on elk farms in Saskatchewan. *The Canadian Veterinary Journal* 48(12): 1241-8.

Arifin, M. I., A. Staskevicius, S.Y. Shim, Y-H. Huang, H. Fenton, P.D. McLoughlin, G. Mitchell, C.I. Cullingham, and S. Gilch. 2020. Large-scale prion protein genotyping in Canadian caribou populations and potential impact on chronic wasting disease susceptibility. *Molecular Ecology* 29 (20):3830-3840. https://doi.org/https://doi.org/10.1111/mec.15602.

Arnot, C., E. Laate, J. Unterschultz, and W. Adamowicz. 2009. Chronic Wasting Disease (CWD) Potential Economic Impact on Cervid Farming in Alberta. Journal of Toxicology and Environmental Health. 72 Part A(17-18):1014-1017. https://doi.org/10.1080/15287390903084223.

Association of Fish & Wildlife Agencies and the Arizona Game and Fish Department. 2017. The State Conservation Machine. https://www.fishwildlife.org/application/files/3615/1853/8699/The_State_ Conservation_Machine-FINAL.pdf.

Atarashi, R., J.M. Wilham, L. Christensen, A.G. Hughson, R.A. Moore, L.M. Johnson, H.A. Onwubiko, S.A. Priola, and B. Caughey. 2008. Simplified ultrasensitive prion detection by recombinant PrP conversion with shaking. *Nature Methods* 5(3):211-2. https://doi.org/10.1038/nmeth0308-211.

Atarashi, R., K. Sano, K. Satoh, and N. Nishida. 2011. Real-time quaking-induced conversion: a highly sensitive assay for prion detection. *Prion* 5(3):150-3. https://doi.org/10.4161/pri.5.3.16893.

Atarashi, R., R.A. Moore, V.L. Sim, A.G. Hughson, D.W. Dorward, H.A. Onwubiko, S.A. Priola, and B. Caughey. 2007. Ultrasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. *Nature Methods* 4(8):645-50. https://doi.org/10.1038/nmeth1066.

Baier, M., S. Norley, J. Schultz, M. Burwinkel, A. Schwarz, and C. Riemer. 2003. Prion diseases: infectious and lethal doses following oral challenge. *Journal of General Virology*. 84(7):1927-1929. https://doi.org/https://doi.org/10.1099/vir.0.19037-0.

Baker, M. 2022. The epidemiology of chronic wasting disease on Saskatchewan cervid farms (2002–2017). MS, Veterinary Pathology, University of Saskatchewan.

Balachandran, A., N.P. Harrington, J. Algire, A. Soutyrine, T.R. Spraker, M. Jeffrey, L. González, and K.I. O'Rourke. 2010. Experimental oral transmission of chronic wasting disease to red deer (Cervus elaphus elaphus): early detection and late-stage distribution of protease-resistant prion protein. *The Canadian Veterinary Journal* 51(2):169-78.

Bartz, J.C. 2021 Environmental and host factors that contribute to prion strain evolution. *Acta Neuropathologica*. 142: 5-16. https://doi.org/10.1007/s00401-021-02310-6.

Bartz, J.C., R.F. Marsh, D.I. McKenzie, and J.M. Aiken. 1998. The host range of chronic wasting disease is altered on passage in ferrets. *Virology*. 251 (2): 297-301. https://doi.org/10.1006/viro.1998.9427.

Bartz, J.C. 2016. Prion Strain Diversity. Cold Spring Harbor Perspectives in Medicine 6(12). https://doi.org/10.1101/cshperspect.a024349.

Bartz, J.C., R.A. Bessen, D. McKenzie, R.F. Marsh, and J.M. Aiken. 2000. Adaptation and Selection of Prion Protein Strain Conformations following Interspecies Transmission of Transmissible Mink Encephalopathy. *Journal of Virology* 74(12):5542-5547. https://doi.org/doi:10.1128/jvi.74.12.5542-5547.2000.

Baune, C., L.L. Wolfe, K.C. Schott, K.A. Griffin, A.G. Hughson, M.W. Miller, and B. Race. 2021. Reduction of Chronic Wasting Disease Prion Seeding Activity following Digestion by Mountain Lions. *mSphere* 6(6):e0081221. https://doi.org/10.1128/msphere.00812-21.

Baylis, M., and W. Goldmann. 2004. The genetics of scrapie in sheep and goats. *Current Molecular Medicine* 4(4):385-96. https://doi.org/10.2174/1566524043360672.

Belay, E.D., P. Gambetti, L.B. Schonberger, P. Parchi, D.R. Lyon, S. Capellari, J.H. McQuiston, K. Bradley, G. Dowdle, J.M. Crutcher, and C.R. Nichols. 2001. Creutzfeldt-Jakob disease in unusually young

patients who consumed venison. *Archives of Neurology* 58(10):1673-8. https://doi.org/10.1001/archneur.58.10.1673.

Belsare, A.V., and C.M. Stewart. 2020. OvCWD: An agent-based modeling framework for informing chronic wasting disease management in white-tailed deer populations. *Ecological Solutions and Evidence*. 1(1):e12017. https://doi.org/10.1002/2688-8319.12017.

Benavente, R., J.H. Reed, M. Lockwood, and R. Morales. 2023. PMCA screening of retropharyngeal lymph nodes in white-tailed deer and comparisons with ELISA and IHC. *Scientific Reports* 13(1):20171. https://doi.org/10.1038/s41598-023-47105-9.

Benestad, S.L., P. Sarradin, B. Thu, J. Schönheit, M.A. Tranulis, and B. Bratberg. 2003. Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. *Veterinary Record* 153(7):202-8. https://doi.org/10.1136/vr.153.7.202.

Benestad, S.L., J.N. Arsac, W. Goldmann, and M. Nöremark. 2008. Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. *Veterinary Research* 39(4):19. https://doi.org/10.1051/vetres:2007056.

Benestad, S.L., L. Austbø, M.A. Tranulis, A. Espenes, and I. Olsaker. 2012. Healthy goats naturally devoid of prion protein. *Veterinary Research* 43(1):87. https://doi.org/10.1186/1297-9716-43-87.

Benestad, S.L., G. Mitchell, M. Simmons, B. Ytrehus, and T. Vikøren. 2016. First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer. *Veterinary Research* 47 (1): 88. https://doi.org/10.1186/s13567-016-0375-4.

Bessen, R.A., and R.F. Marsh. 1992. Biochemical and physical properties of the prion protein from two strains of the transmissible mink encephalopathy agent. *Journal of Virology* 66(4):2096-101. https://doi.org/10.1128/jvi.66.4.2096-2101.1992.

Beyer, W.N., E.E. Connor, and S. Gerould. 1994. Estimates of Soil Ingestion by Wildlife. *The Journal of Wildlife Management* 58(2):375-382. https://doi.org/10.2307/3809405.

Bian, J., D. Napier, V. Khaychuck, R. Angers, C. Graham, and G. Telling. 2010. Cell-based quantification of chronic wasting disease prions. *Journal of Virology* 84(16):8322-6. https://doi.org/10.1128/jvi.00633-10.

Bian, J., J.R. Christiansen, J.A. Moreno, S.J. Kane, V. Khaychuk, J. Gallegos, S. Kim, and G.C. Telling. 2019. Primary structural differences at residue 226 of deer and elk PrP dictate selection of distinct CWD prion strains in gene-targeted mice. *Proceedings of the National Academy of Sciences* 116(25):12478-12487. https://doi.org/doi:10.1073/pnas.1903947116.

Bian, J., S. Kim, S.J. Kane, J. Crowell, J.L. Sun, J. Christiansen, E. Saijo, J.A. Moreno, J. DiLisio, E. Burnett, S. Pritzkow, D. Gorski, C. Soto, T.J. Kreeger, A. Balachandran, G. Mitchell, M.W. Miller, R. Nonno, T. Vikøren, J. Våge, K. Madslien, L. Tran, T.T. Vuong, S. L. Benestad, and G.C. Telling. 2021. Adaptive selection of a prion strain conformer corresponding to established North American CWD during propagation of novel emergent Norwegian strains in mice expressing elk or deep prion protein. *PLOS Pathogen* 17(7):e1009748. https://doi.org/10.1371/journal.ppat.1009748.

Bishop, R.C. 2004. The Economic Impacts of Chronic Wasting Disease (CWD) in Wisconsin. Human Dimensions of Wildlife 9(3):181-192, https://doi.org/10.1080/10871200490479963.

Blanchong, J.A., D.A. Grear, B.V. Weckworth, D.P. Keane, K.T. Scribner, and M.D. Samuel. 2012. Effects of chronic wasting disease on reproduction and fawn harvest vulnerability in Wisconsin white-tailed deer. *Journal of Wildlife Diseases* 48(2):361-70. https://doi.org/10.7589/0090-3558-48.2.361.

Blättler, T., S. Brandner, A. J. Raeber, M. A. Klein, T. Voigtländer, C. Weissmann, and A. Aguzzi. 1997. PrP-expressing tissue required for transfer of scrapie infectivity from spleen to brain. *Nature* 389(6646): 69-73. https://doi.org/10.1038/37981.

Block, A.J. and J.C. Bartz. 2023. Prion strains: shining new light on old concepts. *Cell and Tissue Research* 392:113-133. https://doi.org/10.1007/s00441-022-03665-2.

Boden, L., I. Handel, N. Hawkins, F. Houston, H. Fryer, and R. Kao. 2012. An Economic Evaluation of Preclinical Testing Strategies Compared to the Compulsory Scrapie Flock Scheme in the Control of Classical Scrapie. *PLOS ONE* 7(3):e32884, https://doi.org/10.1371/journal.pone.0032884.

Boden, L.A., F. Houston, H.R. Fryer, and R.R. Kao. 2010. Use of a preclinical test in the control of classical scrapie. *Journal of General Virology* 91(Pt 10):2642-50. https://doi.org/10.1099/vir.0.022566-0.

Bollinger, T., P. Caley, E. Merrill, F. Messier, M. Miller, M. Samuel, and E. Vanopdenbosch. 2004. Chronic Wasting Disease in Canadian Wildlife: An Expert Opinion on the Epidemiology and Risks to Wild

Deer, Expert Scientific Panel on Chronic Wasting Disease. Canadian Cooperative Wildlife Health Centre: Newsletters & Publications 19.

Bolton, D.C., M.P. McKinley, and S.B. Prusiner. 1982. Identification of a protein that purifies with the scrapie prion. *Science* 218(4579):1309-11. https://doi.org/10.1126/science.6815801.

Brandt, A.L., A. C. Kelly, M.L. Green, P. Shelton, J. Novakofski, and N.E. Mateus-Pinilla. 2015. Prion protein gene sequence and chronic wasting disease susceptibility in white-tailed deer (Odocoileus virginianus). *Prion* 9(6):449-462, https://doi.org/10.1080/19336896.2015.1115179.

Bravo-Risi, F., P. Soto, T. Eckland, R. Dittmar, S. Ramírez, C.S.G. Catumbela, C. Soto, M. Lockwood, T. Nichols, and R. Morales. 2021. Detection of CWD prions in naturally infected white-tailed deer fetuses and gestational tissues by PMCA. *Scientific Reports* 11(1):18385. https://doi.org/10.1038/s41598-021-97737-y.

Brooks, J.W., and B.M. Jayarao. 2008. Management practices used by white-tailed deer farms in Pennsylvania and herd health problems. *Journal of the American Veterinary Medical Association* 232(1):98-104. https://doi.org/10.2460/javma.232.1.98.

Brown, Paul, Edward H. Rau, Paul Lemieux, Bruce K. Johnson, Alfred E. Bacote, and D. Carleton Gajdusek. 2004. Infectivity Studies of Both Ash and Air Emissions from Simulated Incineration of Scrapie-Contaminated Tissues. *Environmental Science & Technology* 38(22):6155-6160. https://doi.org/10.1021/es040301z.

Brown, T.L., D.J. Decker, J.T. Major, P.D. Curtis, J.E. Shanahan, and W.F. Siemer. 2006. Hunters' and Other Citizens' Reactions to Discovery of CWD in Central New York. *Human Dimensions of Wildlife* 11(3):203-214. https://doi.org/10.1080/10871200600669924.

Brown, T.L., Decker, D.J., Major, J.T., and W.H. Gordon. 2006. Managing Chronic Wasting Disease in Oneida County, New York: Assessment of Landowners. Human Dimensions Research Unit (HDRU) in the Department of Natural Resources at Cornell University. HDRU Series No 06-4. https://ecommons.cornell.edu/server/api/core/bitstreams/b8e37403-53ca-47ea-aed8-01428c46f5ec/content (accessed August 27, 2024).

Browning, S.R., G.L. Mason, T. Seward, M. Green, G.A. Eliason, C. Mathiason, M.W. Miller, E.S. Williams, E. Hoover, and G.C. Telling. 2004. Transmission of prions from mule deer and elk with chronic wasting disease to transgenic mice expressing cervid PrP. *Journal of Virology* 78(23):13345-50. https://doi.org/10.1128/jvi.78.23.13345-13350.2004.

Bruce, M.E. 1993. Scrapie strain variation and mutation. *British Medical Bulletin* 49(4):822-38. https://doi.org/10.1093/oxfordjournals.bmb.a072649.

Bruce, M.E., R.G. Will, J.W. Ironside, I. McConnell, D. Drummond, A. Suttie, L. McCardle, A. Chree, J. Hope, C. Birkett, S. Cousens, H. Fraser, and C.J. Bostock. 1997. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 389(6650):498-501. https://doi.org/10.1038/39057.

Büeler, H., A. Aguzzi, A. Sailer, R.A. Greiner, P. Autenried, M. Aguet, and C. Weissmann. 1993. Mice devoid of PrP are resistant to scrapie. *Cell* 73(7):1339-1347. https://doi.org/10.1016/0092-8674(93)90360-3.

Burgener, K.R., S.S. Lichtenberg, A. Lomax, D. J. Storm, D.P. Walsh, and J.A. Pedersen. 2022. Diagnostic testing of chronic wasting disease in white-tailed deer (Odocoileus virginianus) by RT-QuIC using multiple tissues. *PLOS ONE* 17(11):e0274531. https://doi.org/10.1371/journal.pone.0274531.

Burton, M., and T. Young. 1996. The impact of BSE on the demand for beef and other meats in Great Britain. *Applied Economics* 28(6):687-693. https://doi.org/10.1080/000368496328434.

Cannon, R.M., and R.T. Roe. 1982. (Richard Treloar) & Australian Bureau of Animal Health. Epidemiology Branch. *Livestock Disease Surveys: A Field Manual for Veterinarians*. Bureau of Rural Science, Department of Primary Industry. Canberra, Australia.

Carlson, C.M., J.R. Schneider, J.A. Pedersen, D.M. Heisey, and C.J. Johnson. 2015. Experimental infection of meadow voles (Microtus pennsylvanicus) with sheep scrapie. *Canadian Journal of Veterinary Research* 79(1):68-73.

Carlson, C.M., S. Thomas, M.W. Keating, P. Soto, N.M. Gibbs, H. Chang, J.K. Wiepz, A.G. Austin, J.R. Schneider, R. Morales, C.J. Johnson, and J.A. Pedersen. 2023. Plants as vectors for environmental prion transmission. *iScience* 26(12):108428. https://doi.org/10.1016/j.isci.2023.108428.

Carroll JA, Chesebro B. 2019. Neuroinflammation, Microglia, and Cell-Association during Prion Disease. *Viruses* 11(1):65. https://doi.org/10.3390/v11010065.

Carter, D.O., D. Yellowlees, and M. Tibbett. 2007. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften* 94(1):12-24. https://doi.org/10.1007/s00114-006-0159-1.

Casalone, C., G. Zanusso, P. Acutis, S. Ferrari, L. Capucci, F. Tagliavini, S. Monaco, and M. Caramelli. 2004. Identification of a second bovine amyloidotic spongiform encephalopathy: Molecular similarities with sporadic Creutzfeldt-Jakob disease. *Proceedings of the National Academy of Sciences* 101(9):3065-3070. https://doi.org/doi:10.1073/pnas.0305777101.

Casellas Connors, J. P., and C.M. Rea. 2022. Violent Entanglements: The Pittman-Robertson Act, Firearms, and the Financing of Conservation. *Conservation and Society* 20(1):24-35. https://doi.org/10.4103/cs.cs_82_21.

Cassmann, E.D., A.J. Frese, S.J. Moore, and J.J. Greenlee. 2022. Transmission of Raccoon-Passaged Chronic Wasting Disease Agent to White-Tailed Deer. *Viruses* 14(7):1578.

Cassmann, E.D., R.D. Frese, and J.J. Greenlee. 2021. Second passage of chronic wasting disease of mule deer to sheep by intracranial inoculation compared to classical scrapie. *Journal of Veterinary Diagnostic Investigation* 33(4):711-720. https://doi.org/10.1177/10406387211017615.

Castilla, J., P. Saá, and C. Soto. 2005. Detection of Prions in Blood. *Nat Med* 11(9):982-5. https://doi.org/10.1038/nm1286.

Caughey, B.W., A. Dong, K.S. Bhat, D. Ernst, S.F. Hayes, and W.S. Caughey. 1991. Secondary structure analysis of the scrapie-associated protein PrP 27-30 in water by infrared spectroscopy. *Biochemistry*. 30 (31): 7672-7680. https://doi.org/10.1021/bi00245a003.

CDC. 2004. Bovine spongiform encephalopathy in a dairy cow–Washington state, 2003. *Morbidity and Mortality Weekly Report* 52(53):1280-5.

Centers for Disease Control and Prevention (CDC), and National Institutes of Health (NIH). 2020. *Biosafety in Microbiological and Biomedical Laboratories.* Edited by P.J. Meechan and J. Potts. 6th edition. U.S. Department of Health and Human Services. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.

Chafin, T.K., M.R. Douglas, B.T. Martin, Z.D. Zbinden, C.R. Middaugh, and J.R. Ballard. 2020. Age Structuring and Spatial Heterogeneity in Prion Protein Gene (PRNP) Polymorphism in White-Tailed Deer. *Prion* 14(1):238-248. https://doi.org/10.1080/19336896.2020.1832947.

Chang, S.C., M.I. Arifin, W. Tahir, K.J. McDonald, D. Zeng, H.M. Schatzl, S. Hannaoui, and S. Gilch. 2024. Extraneural infection route restricts prion conformational variability and attenuates the impact of quaternary structure on infectivity. *PLoS Pathogen* 20(7):e1012370. https://doi.org/10.1371/journal.ppat.1012370.

Chen, B., R. Morales, M.A. Barria, and C. Soto. 2010. Estimating prion concentration in fluids and tissues by quantitative PMCA. *Nature Methods* 7(7):519-20. https://doi.org/10.1038/nmeth.1465.

Cheng Y.C., S. Hannaoui, T.R. John, S. Dudas, S. Czub, and S. Gilch. 2016. Early and Non-Invasive Detection of Chronic Wasting Disease Prions in Elk Feces by Real-Time Quaking Induced Conversion. *PLOS ONE* 11(11):e0166187. https://doi.org/10.1371/journal.pone.0166187.

Chiavacci, S.J. 2022. The economic costs of chronic wasting disease in the United States. *PLOS ONE* 17(12): e0278366. https://doi.org/10.1371/journal.pone.0278366.

Christenson, P.R., M. Li, G. Rowden, M.D. Schwabenlander, T.M. Wolf, S.H. Oh, and P.A. Larsen. 2022. A field-deployable diagnostic assay for the visual detection of misfolded prions. *Scientific Reports* 12(1):12246. https://doi.org/10.1038/s41598-022-16323-y.

Christenson, P.R., M. Li, G. Rowden, P.A. Larsen, and S-H. Oh. 2023. Nanoparticle-Enhanced RT-QuIC (Nano-QuIC) Diagnostic Assay for Misfolded Proteins. *Nano Letters* 23(9):4074-4081. https://doi.org/10.1021/acs.nanolett.3c01001.

Chronic Wasting Disease Task Force. 2002. *Plan for Assisting States, Federal Agencies, and Tribes in Managing Chronic Wasting Disease in Wild and Captive Cervids June 26, 2002*. https://cwd-info.org/wp-content/uploads/2019/03/Federal-Plan-for-Assisting-States-Federal-Agencies-and-Tribes-2002.pdf.

Coffey, B., J. Mintert, J. Fox, T. Schroeder, and L. Valentin. 2005. The economic impact of BSE on the US beef industry: Product value losses, regulatory costs, and consumer reactions. (Extension Bulletin MF-

2678). Kansas State University Agricultural Experiment Station and Cooperative Extension Service. Manhattan, KS.

Collins, S.J., V. Lewis, M.W. Brazier, A.F. Hill, V.A. Lawson, G.M. Klug, and C.L. Masters. 2005. Extended period of asymptomatic prion disease after low dose inoculation: Assessment of detection methods and implications for infection control. *Neurobiology of Disease* 20(2):336-346. https://doi.org/10.1016/j.nbd.2005.03.014.

Conner, M.M., C.W. McCarty, and M.W. Miller. 2000. Detection of bias in harvest-based estimates of chronic wasting disease prevalence in mule deer. *Journal of Wildlife Diseases* 36(4):691-9. https://doi.org/10.7589/0090-3558-36.4.691.

Conner, M.M., and M.W. Miller. 2004. Movement patterns and spatial epidemiology of a prion disease in mule deer population units. *Ecological Applications* 14:1870-1881.

Conner, M.M., M.E. Wood, A. Hubbs, J. Binfet, A.A. Holland, L.R. Meduna, A. Roug, J.P. Runge, T.D. Nordeen, M.J. Pybus, and M.W. Miller. 2021. The Relationship Between Harvest Management and Chronic Wasting Disease Prevalence Trends in Western Mule Deer (Odocoileus hemionus) Herds. *Journal of Wildlife Diseases* 57(4):831-843. https://doi.org/10.7589/jwd-d-20-00226.

Conner, M.M., M.W. Miller, M.R. Ebinger, and K.P. Burnham. 2007. A meta-BACI approach for evaluating management intervention on chronic wasting disease in mule deer. *Journal of Applied Ecology* 17(1):140-53. https://doi.org/10.1890/1051-0761(2007)017[0140:amafem]2.0.co;2.

Cooney, E.E. 2008. The Role of Risk Perceptions in Hunter Support for Deer Density Reduction as a Chronic Wasting Disease (CWD) Management Strategy in Wisconsin. Master's Thesis. University of Wisconsin, Stevens Point, WI. https://epapers.uwsp.edu/thesis/2008/Cooney.pdf.

Cooney, E.E., and R.H. Holsman. 2010. Influences on Hunter Support for Deer Herd Reduction as a Chronic Wasting Disease (CWD) Management Strategy. *Human Dimensions of Wildlife* 15(3):194-207. https://doi.org/10.1080/10871201003598785.

Cooper, S.K., C.E. Hoover, D.M. Henderson, N.J. Haley, C.K. Mathiason, and E.A. Hoover. 2019. Detection of CWD in cervids by RT-QuIC assay of third eyelids. *PLoS ONE* 14(8):e0221654. https://doi.org/10.1371/journal.pone.0221654.

Courtney, S.E. 2023. Factors Affecting Chronic Wasting Disease Prion Transmission Among White-Tailed Deer (Odocoileus Virginianus) in Southern Michigan. Master of Science, Fisheries and Wildlife, Michigan State University.

Cullingham, C.I., R.M. Peery, A. Dao, D. I. McKenzie, and D.W. Coltman. 2020. Predicting the spread-risk potential of chronic wasting disease to sympatric ungulate species. *Prion* 14:56-66. https://doi.org/10.1080/19336896.2020.1720486.

Czub, S., W. Schulz-Schaeffer, C. Stahl-Hennig, M. Beekes, H. and Schaetzl, and D. Motzkus. 2017. First evidence of intracranial and peroral transmission of chronic wasting disease (CWD) into Cynomolgus macaques: A work in progress. *Prion.*

Darish, J.R., A.W. Kaganer, B.J. Hanley, K.L. Schuler, M.D. Schwabenlander, T.M. Wolf, M.S. Ahmed, G.R. Rowden, P.A. Larsen, E. Kobashigawa, D. Tewari, S. Lichtenberg, J.A. Pedersen, S. Zhang, and S. Sreevatsan. Inter-laboratory comparison of real-time quaking-induced conversion (RT-QuIC) for the detection of chronic wasting disease prions in the white-tailed deer retropharyngeal lymph nodes. *Journal of Veterinary Diagnostic Investigation.* https://doi.org/10.1177/10406387241285165.

Davenport, K.A., D.M. Henderson, J. Bian, G.C. Telling, C.K. Mathiason, and E.A. Hoover. 2015. Insights into Chronic Wasting Disease and Bovine Spongiform Encephalopathy Species Barriers by Use of Real-Time Conversion. *Journal of Virology* 89(18):9524-31. https://doi.org/10.1128/jvi.01439-15.

Decker, D.J., S.J. Riley, and W.F. Siemer, eds. *Human dimensions of wildlife management.* JHU Press. 2012.

Deleault, N.R., B.T. Harris, J.R. Rees, and S. Supattapone. 2007. Formation of native prions from minimal components in vitro. *Proceedings of the National Academy of Sciences of the United States of America* 104(23):9741-6. https://doi.org/10.1073/pnas.0702662104.

Demirjian, C., F. Vailleau, R. Berthomé, and F. Roux. 2023. Genome-wide association studies in plant pathosystems: success or failure? *Trends in Plant Science* 28(4):471-485. https://doi.org/10.1016/j.tplants.2022.11.006.

Denkers, N.D., G.C. Telling, and E.A. Hoover. 2011. Minor oral lesions facilitate transmission of chronic wasting disease. *Journal of Virology* 85(3):1396-9. https://doi.org/10.1128/jvi.01655-10.

Denkers, N.D., J. Hayes-Klug, K.R. Anderson, D.M. Seelig, N.J. Haley, S.J. Dahmes, D.A. Osborn, K.V. Miller, R.J. Warren, C.K. Mathiason, and E.A. Hoover. 2013. Aerosol transmission of chronic wasting disease in white-tailed deer. *Journal of Virology* 87(3):1890-2. https://doi.org/10.1128/jvi.02852-12.

Denkers, N.D., D.M. Henderson, C.K. Mathiason, and E.A. Hoover. 2016. Enhanced prion detection in biological samples by magnetic particle extraction and real-time quaking-induced conversion. *Journal of General Virology* 97(8):2023-2029. https://doi.org/10.1099/jgv.0.000515.

Denkers, N.D., C.E. Hoover, K.A. Davenport, D.M. Henderson, E.E. McNulty, A.V. Nalls, C.K. Mathiason, and E.A. Hoover. 2020. Very low oral exposure to prions of brain or saliva origin can transmit chronic wasting disease. *PLOS ONE* 15(8):e0237410. https://doi.org/10.1371/journal.pone.0237410.

Denkers, N. D., McNulty, E. E., Kraft, C. N., Nalls, A. V., Westrich, J. A., Hoover, E. A....Mathiason, C. K. (2024). Temporal Characterization of Prion Shedding in Secreta of White-Tailed Deer in Longitudinal Study of Chronic Wasting Disease, United States. *Emerging Infectious Diseases* 30(10):2118-2127. https://doi.org/10.3201/eid3010.240159.

Detwiler, L.A., and R. Rubenstein. 2000. Bovine spongiform encephalopathy: an overview. *ASAIO Journal* 46(6):S73-9. https://doi.org/10.1097/00002480-200011000-00041.

DeVivo, M.T., D.R. Edmunds, M.J. Kauffman, B.A. Schumaker, J. Binfet, T.J. Kreeger, B.J. Richards, H.M. Schätzl, and T.E. Cornish. 2017. Endemic chronic wasting disease causes mule deer population decline in Wyoming. *PLOS ONE* 12(10):e0186512. https://doi.org/10.1371/journal.pone.0186512.

Di Bari, M.A., R. Nonno, J. Castilla, C. D'Agostino, L. Pirisinu, G. Riccardi, M. Conte, J. Richt, R. Kunkle, J. Langeveld, G. Vaccari, and U. Agrimi. 2013. Chronic wasting disease in bank voles: characterisation of the shortest incubation time model for prion diseases. *PLoS Pathogen* 9(3):e1003219. https://doi.org/10.1371/journal.ppat.1003219.

Dicker, R.C. 1992. *Principles of Epidemiology*. Atlanta, GA: Centers for Disease Control. https://stacks.cdc.gov/view/cdc/11200/cdc_11200_DS1.pdf.

Didier, A., M. Bourner, G. Kleks, A. Zolty, B. Kumar, T. Nichols, K. Durynski, S. Bender, M. Gibison, J.C. Ellis, D.W. Dong, and A. Kashina. 2024. Prospective fecal microbiomic biomarkers for chronic wasting disease. *Microbiology Spectrum* 0(0):e03750-22. https://doi.org/doi:10.1128/spectrum.03750-22.

Diefenbach, D.R., C.S. Rosenberry, and R.C. Boyd. 2004. From the field: efficacy of detecting chronic wasting disease via hunter-killed white-tailed deer. *Wildlife Society Bulletin* 32:267–272. https://doi.org/10.2193/0091-7648(2004)32[267:FTFEOD]2.0.CO;2

Ding, Y., M.M. Veeman, and W.L. Adamowicz. 2011. Habit, BSE, and the Dynamics of Beef Consumption. *Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie* 59(3):337-359. https://doi.org/10.1111/j.1744-7976.2010.01205.x.

Dobbin, M.A., P. Smolko, L. Put, and E.H. Merrill. 2023. Risky business: relating probability of direct contact to risk of chronic wasting disease. *Frontiers in Ecology and Evolution* 11. https://doi.org/10.3389/fevo.2023.1156853.

Dohoo, I., W. Martin, and H. Stryhn. 2014. *Veterinary Epidemiologic Research*. 2nd ed. VER Inc.

Donatuto, J.L., T.A. Satterfield, and R. Gregory. 2011. Poisoning the body to nourish the soul: Prioritising health risks and impacts in a Native American community. *Health, Risk & Society* 13(2):103-127. https://doi.org/10.1080/13698575.2011.556186.

Donoghue, E.M., S.A. Thompson, and J.C. Bliss. 2010. Tribal-Federal Collaboration in Resource Management. *Journal of Ecological Anthropology* 14(1):22-38.

Dorak, S.J., M.L. Green, M.M. Wander, M.O. Ruiz, M.G. Buhnerkempe, T. Tian, J.E. Novakofski, and N.E. Mateus-Pinilla. 2017. Clay content and pH: soil characteristic associations with the persistent presence of chronic wasting disease in northern Illinois. *Scientific Reports* 7(1):18062. https://doi.org/10.1038/s41598-017-18321-x.

Dubé, C., K.G. Mehren, I.K. Barker, B.L. Peart, and A. Balachandran. 2006. Retrospective investigation of chronic wasting disease of cervids at the Toronto Zoo, 1973-2003. *The Canadian Veterinary Journal* 47(12):1185-93.

Dulberger, J., N.T. Hobbs, H.M. Swanson, C.J. Bishop, and M.W. Miller. 2010. Estimating chronic wasting disease effects on mule deer recruitment and population growth. *Journal of Wildlife Diseases* 46(4):1086-95. https://doi.org/10.7589/0090-3558-46.4.1086.

Duque Velásquez, C., C. Kim, A. Herbst, N. Daude, M.C. Garza, H. Wille, J. Aiken, and D. McKenzie. 2015. Deer Prion Proteins Modulate the Emergence and Adaptation of Chronic Wasting Disease Strains. *Journal of Virology* 89(24):12362-73. https://doi.org/10.1128/jvi.02010-15.

Duque Velásquez, C., C. Kim, T. Haldiman, C. Kim, A. Herbst, J. Aiken, J.G. Safar, and D. McKenzie. 2020. Chronic wasting disease (CWD) prion strains evolve via adaptive diversification of conformers in hosts expressing prion protein polymorphisms. *Journal of Biological Chemistry* 295(15):4985-5001. https://doi.org/10.1074/jbc.RA120.012546.

Edmunds, D.R., M.J. Kauffman, B.A. Schumaker, F.G. Lindzey, W.E. Cook, T.J. Kreeger, R.G. Grogan, and T.E. Cornish. 2016. Chronic Wasting Disease Drives Population Decline of White-Tailed Deer. *PLOS ONE*. 11(8):e0161127. https://doi.org/10.1371/journal.pone.0161127.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention Control). 2014. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012. *EFSA Journal* 12(2):3547. https://doi.org/10.2903/j.efsa.2014.3547.

EFSA Panel on Biological Hazards, Koutsoumanis K., A. Allende, A. Alvarez-Ordonez, D. Bolton, S. Bover-Cid, M. Chemaly, R. Davies, A. De Cesare, L. Herman, F. Hilbert, R. Lindqvist, M. Nauta, L. Peixe, G. Ru, P. Skandamis, E. Suffredini, O. Andreoletti, S.L. Benestad, E. Comoy, R. Nonno, T. da Silva Felicio, A. Ortiz-Pelaez, and M.M. Simmons. 2019. Scientific opinion on the update on chronic wasting disease (CWD) III. *EFSA Journal* 17(11):5863. https://doi.org/ 10.2903/j.efsa.2019.5863.

EFSA Panel on Biological Hazards, K. Koutsoumanis, A. Allende, A. Alvarez-Ordoñez, D. Bolton, S. Bover-Cid, M. Chemaly, R. Davies, A. De Cesare, L. Herman, F. Hilbert, R. Lindqvist, M. Nauta, L. Peixe, P. Skandamis, E. Suffredini, M.W. Miller, A. Mysterud, M. Nöremark, M. Simmons, M.A. Tranulis, G. Vaccari, H. Viljugrein, A. Ortiz-Pelaez, and G. Ru. 2023. Monitoring of chronic wasting disease (CWD) (IV). *EFSA Journal* 21(4):e07936. https://doi.org/https://doi.org/10.2903/j.efsa.2023.7936.

EFSA Panel on Biological Hazards, A. Ricci, A. Allende, D. Bolton, M. Chemaly, R. Davies, P. Fernández Escámez, R. Salvador Gironés, L. Herman, K. Koutsoumanis, R. Lindqvist, B. Nørrung, L. Robertson, G. Ru, M. Sanaa, P. Skandamis, E. Snary, N. Speybroeck, B.T. Kuile, J. Threlfall, H. Wahlström, S. Benestad, D. Gavier-Widen, M.W. Miller, G.C. Telling, M. Tryland, F. Latronico, A. Ortiz-Pelaez, P. Stella, and M. Simmons. 2018. Scientific opinion on chronic wasting disease (II). *EFSA Journal* 16(1):e05132. https://doi.org/10.2903/j.efsa.2018.5132.

EFSA Panel on Biological Hazards. 2017. Chronic wasting disease (CWD) in cervids. *EFSA Journal* 15(1):e04667. https://doi.org/https://doi.org/10.2903/j.efsa.2017.4667.

Egan, M.E., K.M. Pepin, J.W. Fischer, S.E. Hygnstrom, K.C. VerCauteren, and G. Bastille-Rousseau. 2023. Social network analysis of white-tailed deer scraping behavior: Implications for disease transmission. *Ecosphere* 14(2):e4434. https://doi.org/10.1002/ecs2.4434.

Erickson, D., C. Reeling, and J.G. Lee. 2019. The Effect of Chronic Wasting Disease on Resident Deer Hunting Permit Demand in Wisconsin. *Animals (Basel)*. 9 (12). https://doi.org/10.3390/ani9121096.

Evans, T.S., K.L. Schuler, and W.D. Walter. 2014. Surveillance and monitoring of white-tailed deer for chronic wasting disease in the northeastern United States. *Journal of Fish and Wildlife Management* 5(2):387-393. https://doi.org/10.3996/032014-JFWM-021.

Evans, T.S., M.S. Kirchgessner, B. Eyler, C.W. Ryan, and W.D. Walter. 2015. Habitat influences distribution of chronic wasting disease in white-tailed deer. *Journal of Wildlife Management* 80(2):284-291. https://doi.org/10.1002/jwmg.1004.

Evans, T.S., M.S. Kirchgessner, B. Eyler, C.W. Ryan, and W.D. Walter. 2016. Habitat influences distribution of chronic wasting disease in white-tailed deer. *The Journal of Wildlife Management* 80(2):284-291. https://doi.org/10.1002/jwmg.1004.

Fameli, A.F., J. Edson, J.E. Banfield, C.S. Rosenberry, and W.D. Walter. 2022. Variability in prion protein genotypes by spatial unit to inform susceptibility to chronic wasting disease. *Prion* 16(1):254-264. https://doi.org/10.1080/19336896.2022.2117535.

Farnsworth, M.L., J.A. Hoeting, N.T. Hobbs, and M.W. Miller. 2006. Linking Chronic Wasting Disease to Mule Deer Movement Scales: A Hierarchical Bayesian Approach. *Ecological Applications* 16(3):1026-1036. https://doi.org/10.1890/1051-0761(2006)016[1026:LCWDTM]2.0.CO;2.

Faust, R., T. Wolf, D. Fulton, L. Bernstein, M. Schwabenlander, K. Applegate, A. Ayres, P. May, A. Vig, M. Struck, and C. Yoder. 2023. Using a Community-Based Approach to Develop CWD Outreach with Tribal Communities. Pathways: Human Dimensions of Conservation, Fort Collins, CO, United States.

Faust, R., T. Wolf, D. Fulton, L. Bernstein, M. Schwabenlander, K. Applegate, A. Ayres, P. May, A. Vig, M. Struck, and C. Yoder. 2024. A Community-Based Participatory Approach to Develop Chronic Wasting Disease Outreach and Management with Tribal Communities. 84th Midwest Fish and Wildlife Conference.

Ferreira, N.C., J.M. Charco, J. Plagenz, C.D. Orru, N.D. Denkers, M.A. Metrick, A.G. Hughson, K.A. Griffin, B. Race, E.A. Hoover, J. Castilla, T.A. Nichols, M.W. Miller, and B. Caughey. 2021. Detection of chronic wasting disease in mule and white-tailed deer by RT-QuIC analysis of outer ear. *Scientific Reports* 11(1):7702. https://doi.org/10.1038/s41598-021-87295-8.

Ferreira Caceres, M.M., J.P. Sosa, J.A. Lawrence, C. Sestacovschi, A. Tidd-Johnson, M.H.U. Rasool, V.K. Gadamidi, S. Ozair, K. Pandav, C. Cuevas-Lou, M. Parrish, I. Rodriguez, and J.P. Fernandez. 2022. The impact of misinformation on the COVID-19 pandemic. *AIMS Public Health* 9(2):262-277. https://doi.org/10.3934/publichealth.2022018.

Fischer, J.R., and M. Dunfee. 2022. Chronic Wasting Disease Detection and Management: What Has Worked and What Has Not? A Multi-State Conservation Grant. Technical Report presented to the Association of Fish and Wildlife Agencies. https://cwd-info.org/wp-content/uploads/2022/11/CWD-Dectection-and-Management.pdf

Fischer, J.W., G.E. Phillips, T.A. Nichols, and K.C. VerCauteren. 2013. Could avian scavengers translocate infectious prions to disease-free areas initiating new foci of chronic wasting disease? *Prion* 7(4):263-266. https://doi.org/10.4161/pri.25621.

Fisher, M.C., R.A. Prioreschi, L.L. Wolfe, J.P. Runge, K.A. Griffin, H.M. Swanson, and M.W. Miller. 2022. Apparent stability masks underlying change in a mule deer herd with unmanaged chronic wasting disease. *Communications Biology* 5(1):15. https://doi.org/10.1038/s42003-021-02951-z.

Foley, A.M., D.G. Hewitt, C.A. DeYoung, R.W. DeYoung, and M.J. Schnupp. 2016. Modeled Impacts of Chronic Wasting Disease on White-Tailed Deer in a Semi-Arid Environment. *PLOS ONE* 11(10):e0163592. https://doi.org/10.1371/journal.pone.0163592.

Fox, K.A., J.E. Jewell, E.S. Williams, and M.W. Miller. 2006. Patterns of PrPCWD accumulation during the course of chronic wasting disease infection in orally inoculated mule deer (Odocoileus hemionus). *Journal of General Virology* 87(Pt 11):3451-3461. https://doi.org/10.1099/vir.0.81999-0.

Fox, K.A., S.M. Muller, T.R. Spraker, M.E. Wood, and M.W. Miller. 2021. Opportunistic Surveillance of Captive And Free-Ranging Bighorn Sheep (Ovis Canadensis) in Colorado, USA, for Transmissible Spongiform Encephalopathies. *Journal of Wildlife Diseases* 57(2):338-344. https://doi.org/10.7589/jwd-d-20-00083.

Francisco, T. 2023. Examples of how cervids are valued by Native Americans.

Fraser, H., and A.G. Dickinson. 1967. Distribution of experimentally induced scrapie lesions in the brain. *Nature* 216(5122):1310-1. https://doi.org/10.1038/2161310a0.

Fraser, H., and A.G. Dickinson. 1968. The sequential development of the brain lesion of scrapie in three strains of mice. *Journal of Comparative Pathology* 78(3):301-11. https://doi.org/10.1016/0021-9975(68)90006-6.

Freeman III, A.M., J.A. Herriges, and C.L. Kling. 2014. The Measurement of Environmental and Resource Values: Theory and Methods, 3rd ed. Routledge.

Fryer, H.R., and A.R. McLean. 2011. There is no safe dose of prions. *PLOS ONE* 6(8):e23664. https://doi.org/10.1371/journal.pone.0023664.

Gagnier, M., I. Laurion, and A.J. DeNicola. 2020. Control and Surveillance Operations to Prevent Chronic Wasting Disease Establishment in Free-Ranging White-Tailed Deer in Québec, Canada. *Animals* 10(2):283. https://doi.org/10.3390/ani10020283.

Galloway, N.L., R.J. Monello, D. Brimeyer, E.K. Cole, and N.T. Hobbs. 2021. Supporting adaptive management with ecological forecasting: chronic wasting disease in the Jackson Elk Herd. *Ecosphere* 12(10):e03776. https://doi.org/10.1002/ecs2.3776.

Gambetti, P., Z. Dong, J. Yuan, X. Xiao, M. Zheng, A. Alshekhlee, R. Castellani, M. Cohen, M.A. Barria, D. Gonzalez-Romero, E.D. Belay, L.B. Schonberger, K. Marder, C. Harris, J.R. Burke, T. Montine, T.

Wisniewski, D.W. Dickson, C. Soto, C.M. Hulette, J.A. Mastrianni, Q. Kong, and W.Q. Zou. 2008. A novel human disease with abnormal prion protein sensitive to protease. *Annals of Neurology* 63(6):697-708. https://doi.org/10.1002/ana.21420.

Garruto, Ralph M., Chris Reiber, Marta P. Alfonso, Heidi Gastrich, Kelsey Needham, Sarah Sunderman, Sarah Walker, Jennifer Weeks, Nicholas DeRosa, Eric Faisst, John Dunn, Kenneth Fanelli, and Kenneth Shilkret. 2008. Risk behaviors in a rural community with a known point-source exposure to chronic wasting disease. *Environmental Health* 7(1):31. https://doi.org/10.1186/1476-069X-7-31.

Gassett, J. 2019. Uniform Carcass Transport Rules Could Help Slow the Spread of Chronic Wasting Disease. *Conservation Briefs*. *Wildlife Management Institute* 73(1).

Geist, V., D. Clausen, V. Crichton, and D. Rowledge. 2017. The Challenge of CWD: Insidious and Dire. *Alliance for Public Wildlife*. https://www.apwildlife.org/s/CWD-Comprehensive-Analysis-LR-SP.pdf (accessed November 8, 2024).

Georgsson, G., S. Sigurdarson, and P. Brown. 2006. Infectious agent of sheep scrapie may persist in the environment for at least 16 years. *Journal of General Virology* 87(12):3737-3740. https://doi.org/10.1099/vir.0.82011-0.

Geremia, C., J.A. Hoeting, L.L. Wolfe, N.L. Galloway, M.F. Antolin, T.R. Spraker, M.W. Miller, and N.T. Hobbs. 2015. Age and Repeated Biopsy Influence Antemortem Prp (CWD) Testing in Mule Deer (Odocoileus Hemionus) In Colorado, USA. *Journal of Wildlife Diseases* 51(4):801-10. https://doi.org/10.7589/2014-12-284.

Gigliotti, L.M. 2004. Hunters' Concerns About Chronic Wasting Disease in South Dakota. *Human Dimensions in Wildlife* 9(3):233-235. https://doi.org/10.1080/10871200490480006.

Gilbertson, M.L.J., E.E. Brandell, M.E. Pinkerton, N.M. Meaux, M. Hunsaker, D. Jarosinski, W. Ellarson, D.P. Walsh, D.J. Storm, and W.C. Turner. 2022. Cause of Death, Pathology, and Chronic Wasting Disease Status of White-tailed Deer (Odocoileus virginianus) Mortalities in Wisconsin, USA. *Journal of Wildlife Diseases* 58(4):803-815. https://doi.org/10.7589/jwd-d-21-00202.

Gilch, S., F. Wopfner, I. Renner-Müller, E. Kremmer, C. Bauer, E. Wolf, G. Brem, M.H. Groschup, and H.M. Schätzl. 2003. Polyclonal Anti-PrP Auto-antibodies Induced with Dimeric PrP Interfere Efficiently with PrPSc Propagation in Prion-infected Cells. *Journal of Biological Chemistry* 278(20):18524-18531. https://doi.org/10.1074/jbc.M210723200.

Gill, O.N., Y. Spencer, A. Richard-Loendt, C. Kelly, D. Brown, K. Sinka, N. Andrews, R. Dabaghian, M. Simmons, P. Edwards, P. Bellerby, D.J. Everest, M. McCall, L.M. McCardle, J. Linehan, S. Mead, D.A. Hilton, J.W. Ironside, and S. Brandner. 2020. Prevalence in Britain of abnormal prion protein in human appendices before and after exposure to the cattle BSE epizootic. *Acta Neuropathology* 139(6):965-976. https://doi.org/10.1007/s00401-020-02153-7.

Gill, O.N., Y. Spencer, A. Richard-Loendt, C. Kelly, R. Dabaghian, L. Boyes, J. Linehan, M. Simmons, P. Webb, P. Bellerby, N. Andrews, D.A. Hilton, J.W. Ironside, J. Beck, M. Poulter, S. Mead, and S. Brandner. 2013. Prevalent abnormal prion protein in human appendixes after bovine spongiform encephalopathy epizootic: large scale survey. *British Medical Journal* 347:f5675. https://doi.org/10.1136/bmj.f5675.

Gillin, C.M. 2022. "Health Protection and Promotion for Disease Management in Free-Ranging Wildlife Populations." In *Wildlife Population Health*, edited by C. Stephen, 113-125. Cham, Switzerland: Springer Nature Switzerland AG.

Gillin, C.M. and J.R. Mawdsley (eds). 2018. *AFWA Technical Report on Best Management Practices for Surveillance, Management and Control of Chronic Wasting Disease*. Association of Fish and Wildlife Agencies, Washington, D. C.

Gisondi, M.A., R. Barber, J.S. Faust, A. Raja, M.C. Strehlow, L.M. Westafer, and M. Gottlieb. 2022. A Deadly Infodemic: Social Media and the Power of COVID-19 Misinformation. *Journal of Medical Internet Research* 24(2):e35552. https://doi.org/10.2196/35552.

Goldmann, W., N. Hunter, G. Smith, J. Foster, and J. Hope. 1994. PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *Journal of General Virology* 75(Pt 5):989-95. https://doi.org/10.1099/0022-1317-75-5-989.

Gonzalez-Montalban, N., N. Makarava, V.G. Ostapchenko, R. Savtchenk, I. Alexeeva, R.G. Rohwer, and I.V. Baskakov. 2011. Highly Efficient Protein Misfolding Cyclic Amplification. *PLOS Pathogens* 7(2):e1001277. https://doi.org/10.1371/journal.ppat.1001277.

Gonzalez-Romero, D., M. A. Barria, P. Leon, R. Morales, and C. Soto. 2008. Detection of infectious prions in urine. *FEBS Letters* 582(21-22):3161-6. https://doi.org/10.1016/j.febslet.2008.08.003.

Goodman, M., C.A. Porter, J. Czelusniak, S.L. Page, H. Schneider, J. Shoshani, G. Gunnell, and C.P. Groves. 1998. Toward a phylogenetic classification of Primates based on DNA evidence complemented by fossil evidence. *Molecular Phylogenetics and Evolution* 9(3):585-98. https://doi.org/10.1006/mpev.1998.0495.

Gore, M.L., R.S. Wilson, W.F. Siemer, H.W. Hudenko, C.E. Clarke, P.S. Hart., L.A. Maguire, and B.A. Muter. 2009. Application of Risk Concepts to Wildlife Management: Special Issue Introduction. *Human Dimensions of Wildlife* 14(5):301-313. https://doi.org/10.1080/10871200903160944.

Gough, K.C., C.A. Baker, H.A. Simmons, S.A. Hawkins, and B.C. Maddison. 2015. Circulation of prions within dust on a scrapie affected farm. *Veterinary Research* 46(1):40. https://doi.org/10.1186/s13567-015-0176-1.

Gould, D.H., J.L. Voss, M.W. Miller, A.M. Bachand, B.A. Cummings, and A.A. Frank. 2003. Survey of cattle in northeast Colorado for evidence of chronic wasting disease: geographical and high-risk targeted sample. Journal of Veterinary Diagnostic Investigation 15(3):274-7. https://doi.org/10.1177/104063870301500309.

Great Lakes Inter-Tribal Council Snap-Ed Program. 2015. Waawaashkeshi (Deer).

Greenlee, J.J., S.J. Moore, E.D. Cassmann, Z.J. Lambert, R.D. Kokemuller, J.D. Smith, R.A. Kunkle, Q. Kong, and M.H.W. Greenlee. 2023. Characterization of Classical Sheep Scrapie in White-tailed Deer after Experimental Oronasal Exposure. *The Journal of Infectious Diseases* 227(12):1386-1395. https://doi.org/10.1093/infdis/jiac443.

Greenlee, J.J., E.M. Nicholson, J.D. Smith, R.A. Kunkle, and A.N. Hamir. 2012. Susceptibility of cattle to the agent of chronic wasting disease from elk after intracranial inoculation. *Journal of Veterinary Diagnostic Investigation* 24(6):1087-93. https://doi.org/10.1177/1040638712461249.

Groschup M.H., C. Lacroux, A. Buschmann, G. Lühken, J. Mathey, M. Eiden, S. Lugan, C. Hoffmann, J.C. Espinosa, T. Baron, J.M Torres, G. Erhardt, and O. Andreoletti. 2007. Classic scrapie in sheep with the ARR/ARR prion genotype in Germany and France. *Emerging Infection Diseases* 13(8):1201-7. https://doi.org/10.3201/eid1308.070077.

Gross, J.E., and M.W. Miller. 2001. Chronic Wasting Disease in Mule Deer: Disease Dynamics and Control. *The Journal of Wildlife Management* 65(2):205-215. https://doi.org/10.2307/3802899.

Guadagno, A.H., and S.H. Medina. 2023. The manifold role of octapeptide repeats in prion protein assembly. *Peptide Science* 115(2):e24303. https://doi.org/10.1002/pep2.24303.

Grear, D.A., M.D. Samuel, J.A. Langenberg, and D. Keane. 2010. Demographic Patterns and Harvest Vulnerability of Chronic Wasting Disease Infected White-Tailed Deer in Wisconsin. *Journal of Wildlife Management* 70(2):546-553. https://doi.org/10.2193/0022-541X(2006)70[546:DPAHVO]2.0.CO;2.

Groveman, B.R., K. Williams, B. Race, S. Foliaki, T. Thomas, A.G. Hughson, R.O. Walters, W. Zou, and C.L. Haigh. 2024. Lack of Transmission of Chronic Wasting Disease Prions to Human Cerebral Organoids. *Emerging Infectious Diseases* 30(6):1193-1202. https://doi.org/10.3201/eid3006.231568.

Güere, M.E., J. Våge, H. Tharaldsen, S.L. Benestad, T. Vikøren, K. Madslien, P. Hopp, C.M. Rolandsen, K.H. Røed, and M.A. Tranulis. 2020. Chronic wasting disease associated with prion protein gene (PRNP) variation in Norwegian wild reindeer (Rangifer tarandus). *Prion* 14(1):1-10. https://doi.org/10.1080/19336896.2019.1702446.

Güere, M.E., J. Våge, H. Tharaldsen, K.S. Kvie, B.J. Bårdsen, S.L. Benestad, T. Vikøren, K. Madslien, C.M. Rolandsen, M.A. Tranulis, and K.H. Røed. 2022. Chronic wasting disease in Norway-A survey of prion protein gene variation among cervids. *Transboundary and Emerging Diseases* 69(4):e20-e31. https://doi.org/10.1111/tbed.14258.

Guiroy, D.C., E.S. Williams, K.J. Song, R. Yanagihara, and D.C. Gajdusek. 1993. Fibrils in brain of Rocky Mountain elk with chronic wasting disease contain scrapie amyloid. *Acta Neuropathologica Communications* 86(1):77-80. https://doi.org/10.1007/bf00454902.

Guiroy, D.C., E.S. Williams, R. Yanagihara, and D.C. Gajdusek. 1991. Topographic distribution of scrapie amyloid-immunoreactive plaques in chronic wasting disease in captive mule deer (Odocoileus hemionus hemionus). *Acta Neuropathologica Communications* 81(5):475-8. https://doi.org/10.1007/bf00310125.

Gul, H., G. Habib, I.M. Khan, S.U. Rahman, N.M. Khan, H. Wang, N.U. Khan, and Y. Liu. 2022. Genetic resilience in chickens against bacterial, viral and protozoal pathogens. *Frontiers in Veterinary Science* 9. https://doi.org/10.3389/fvets.2022.1032983.

Habib, T.J., E.H. Merrill, M.J. Pybus, and D.W. Coltman. 2011. Modelling landscape effects on density–contact rate relationships of deer in eastern Alberta: Implications for chronic wasting disease. *Ecological Modelling* 222(15):2722-2732. https://doi.org/10.1016/j.ecolmodel.2011.05.007.

Hagenaars T.J., M.B. Melchior, A. Bossers, A. Davidse, B. Engel, and F.G. van Zijderveld. 2010. Scrapie prevalence in sheep of susceptible genotype is declining in a population subject to breeding for resistance. *BMC Veterinary Research* 6. https://doi.org/10.1186/1746-6148-6-25.

Haigh, J.C., and R.J. Hudson. 1993. Farming wapiti and red deer. Mosby St. Louis.

Haley, N.J., C.K. Mathiason, M.D. Zabel, G.C. Telling, and E.A. Hoover. 2009a. Detection of Sub-Clinical CWD Infection in Conventional Test-Negative Deer Long after Oral Exposure to Urine and Feces from CWD+ Deer. *PLOS ONE* 4(11):e7990. https://doi.org/10.1371/journal.pone.0007990.

Haley, N.J., D.M. Seelig, M.D. Zabel, G.C. Telling, and E.A. Hoover. 2009b. Detection of CWD Prions in Urine and Saliva of Deer by Transgenic Mouse Bioassay. *PLOS ONE* 4(3):e4848. https://doi.org/10.1371/journal.pone.0004848.

Haley, N.J., C.K. Mathiason, S. Carver, M. Zabel, G.C. Telling, and E.A. Hoover. 2011. Detection of chronic wasting disease prions in salivary, urinary, and intestinal tissues of deer: potential mechanisms of prion shedding and transmission. *Journal of Virology* 85(13):6309-18. https://doi.org/10.1128/jvi.00425-11.

Haley, N.J., C.K. Mathiason, S. Carver, G.C. Telling, M.D. Zabel, and E.A. Hoover. 2012. Sensitivity of protein misfolding cyclic amplification versus immunohistochemistry in ante-mortem detection of chronic wasting disease. *Journal of General Virology* 93(Pt 5):1141-1150. https://doi.org/10.1099/vir.0.039073-0.

Haley, N.J., A. Van de Motter, S. Carver, D. Henderson, K. Davenport, D.M. Seelig, C. Mathiason, and E. Hoover. 2013. Prion-seeding activity in cerebrospinal fluid of deer with chronic wasting disease. *PLOS ONE* 8(11):e81488. https://doi.org/10.1371/journal.pone.0081488.

Haley, N.J., S. Carver, L.L. Hoon-Hanks, D.M. Henderson, K.A. Davenport, E. Bunting, S. Gray, B. Trindle, J. Galeota, I. LeVan, T. Dubovos, P. Shelton, and E.A. Hoover. 2014. Detection of chronic wasting disease in the lymph nodes of free-ranging cervids by real-time quaking-induced conversion. *Journal of Clinical Microbiology* 52(9):3237-43. https://doi.org/10.1128/jcm.01258-14.

Haley, N.J., C. Siepker, W.D. Walter, B.V. Thomsen, J.J. Greenlee, A.D. Lehmkuhl, and J.A. Richt. 2016a. Antemortem Detection of Chronic Wasting Disease Prions in Nasal Brush Collections and Rectal Biopsy Specimens from White-Tailed Deer by Real-Time Quaking-Induced Conversion. *The Journal of Clinical Microbiology* 54(4):1108-16. https://doi.org/10.1128/jcm.02699-15.

Haley, N.J., C. Siepker, L.L. Hoon-Hanks, G. Mitchell, W.D. Walter, M. Manca, R.J. Monello, J.G. Powers, M.A. Wild, E.A. Hoover, B. Caughey, and J.A. Richt. 2016b. Seeded Amplification of Chronic Wasting Disease Prions in Nasal Brushings and Recto-anal Mucosa-Associated Lymphoid Tissues from Elk by Real-Time Quaking-Induced Conversion. *The Journal of Clinical Microbiology* 54(4):1117-26. https://doi.org/10.1128/jcm.02700-15.

Haley, N.J., and J.A. Richt. 2017. Evolution of Diagnostic Tests for Chronic Wasting Disease, a Naturally Occurring Prion Disease of Cervids. *Pathogens* 6(3). https://doi.org/10.3390/pathogens6030035.

Haley, N.J., R. Rielinger, K.A. Davenport, K. O'Rourke, G. Mitchell, and J.A. Richt. 2017. Estimating chronic wasting disease susceptibility in cervids using real-time quaking-induced conversion. *Journal of General Virology* 98(11):2882-2892. https://doi.org/10.1099/jgv.0.000952.

Haley, N.J., D.M. Henderson, S. Wyckoff, J. Tennant, E.A. Hoover, D. Love, E. Kline, A. Lehmkuhl, and B. Thomsen. 2018. Chronic wasting disease management in ranched elk using rectal biopsy testing. *Prion* 12(2):93-108. https://doi.org/10.1080/19336896.2018.1436925.

Haley, N.J., K. Merrett, A. Buros Stein, D. Simpson, A. Carlson, G. Mitchell, A. Staskevicius, T. Nichols, A.D. Lehmkuhl, and B.V. Thomsen. 2019. Estimating relative CWD susceptibility and disease progression in farmed white-tailed deer with rare PRNP alleles. *PLOS ONE* 14(12):e0224342. https://doi.org/10.1371/journal.pone.0224342.

Haley, N.J., D.M. Henderson, R. Donner, S. Wyckoff, K. Merrett, J. Tennant, E.A. Hoover, D. Love, E. Kline, A.D. Lehmkuhl, and B.V. Thomsen. 2020a. Management of chronic wasting disease in ranched elk: conclusions from a longitudinal three-year study. *Prion* 14(1):76-87. https://doi.org/10.1080/19336896.2020.1724754.

Haley, N.J., R. Donner, D.M. Henderson, J. Tennant, E.A. Hoover, M. Manca, B. Caughey, N. Kondru, S. Manne, A. Kanthasamay, S. Hannaoui, S.C. Chang, S. Gilch, S. Smiley, G. Mitchell, A.D. Lehmkuhl, and B.V. Thomsen. 2020b. Cross-validation of the RT-QuIC assay for the antemortem detection of chronic wasting disease in elk. *Prion* 14(1):47-55. https://doi.org/10.1080/19336896.2020.1716657.

Haley, N.J., D.M. Henderson, K. Senior, M. Miller, and R. Donner. 2021a. Evaluation of Winter Ticks (Dermacentor albipictus) Collected from North American Elk (Cervus canadensis) in an Area of Chronic Wasting Disease Endemicity for Evidence of PrPCWD Amplification Using Real-Time Quaking-Induced Conversion Assay. *mSphere* 6(4). https://doi.org/doi:10.1128/msphere.00515-21.

Haley, N., R. Donner, K. Merrett, M. Miller, and K. Senior. 2021b. Selective Breeding for Disease-Resistant PRNP Variants to Manage Chronic Wasting Disease in Farmed Whitetail Deer. *Genes* 12(9):1396.

Hall, A.J. 2012. Noroviruses: The Perfect Human Pathogens? *The Journal of Infectious Diseases* 205(11):1622-1624. https://doi.org/10.1093/infdis/jis251.

Hamir, A.N., R.C. Cutlip, J.M. Miller, E.S. Williams, M.J. Stack, M.W. Miller, K.I. O'Rourke, and M.J. Chaplin. 2001. Preliminary findings on the experimental transmission of chronic wasting disease agent of mule deer to cattle. *Journal of Veterinary Diagnostic Investigation* 13(1):91-6. https://doi.org/10.1177/104063870101300121.

Hamir, A.N., T. Gidlewski, T.R. Spraker, J.M. Miller, L. Creekmore, M. Crocheck, T. Cline, and K.I. O'Rourke. 2006a. Preliminary Observations of Genetic Susceptibility of elk (*Cervus elaphus nelsoni*) to Chronic Wasting Disease by Experimental Oral Inoculation. *Journal of Veterinary Diagnostic Investigation* 18(1):110-114. https://doi.org/10.1177/104063870601800118.

Hamir, A.N., R.A. Kunkle, R.C. Cutlip, J.M. Miller, E.S. Williams, and J.A. Richt. 2006b. Transmission of chronic wasting disease of mule deer to Suffolk sheep following intracerebral inoculation. *Journal of Veterinary Diagnostic Investigation*. 18 (6): 558-65. https://doi.org/10.1177/104063870601800606.

Hamir, A.N., R.A. Kunkle, J.M. Miller, R.C. Cutlip, J.A. Richt, M.E. Kehrli, Jr., and E.S. Williams. 2007. Age-related lesions in laboratory-confined raccoons (Procyon lotor) inoculated with the agent of chronic wasting disease of mule deer. *Journal of Veterinary Diagnostic Investigation* 19(6):680-6. https://doi.org/10.1177/104063870701900610.

Hamir, A.N., J.A. Richt, J.M. Miller, R.A. Kunkle, S.M. Hall, E.M. Nicholson, K.I. O'Rourke, J.J. Greenlee, and E.S. Williams. 2008. Experimental transmission of chronic wasting disease (CWD) of elk (Cervus elaphus nelsoni), white-tailed deer (Odocoileus virginianus), and mule deer (Odocoileus hemionus hemionus) to white-tailed deer by intracerebral route. *Veterinary Pathology* 45(3):297-306. https://doi.org/10.1354/vp.45-3-297.

Hamir, A.N., J.J. Greenlee, E.M. Nicholson, R.A. Kunkle, J.A. Richt, J.M. Miller, and M. Hall. 2011. Experimental transmission of chronic wasting disease (CWD) from elk and white-tailed deer to fallow deer by intracerebral route: final report. *Canadian Journal of Veterinary Research* 75(2):152-6.

Hannaoui, S., H.M. Schatzl, and S. Gilch. 2017. Chronic wasting disease: Emerging prions and their potential risk. *PLOS Pathogens* 13(11):e1006619. https://doi.org/10.1371/journal.ppat.1006619.

Hannaoui, S., E. Triscott, C. Duque Velásquez, S.C. Chang, M.I. Arifin, I. Zemlyankina, X. Tang, T. Bollinger, H. Wille, D. McKenzie, and S. Gilch. 2021. New and distinct chronic wasting disease strains associated with cervid polymorphism at codon 116 of the Prnp gene. *PLOS Pathogens* 17(7):e1009795. https://doi.org/10.1371/journal.ppat.1009795.

Hannaoui, S., I. Zemlyankina, S.C. Chang, M.I. Arifin, V. Béringue, D. McKenzie, H.M. Schatzl, and S. Gilch. 2022. Transmission of cervid prions to humanized mice demonstrates the zoonotic potential of CWD. *Acta Neuropathologica Communications* 144(4):767-784. https://doi.org/10.1007/s00401-022-02482-9.

Harrington, R.D., T.V. Baszler, K.I. O'Rourke, D.A. Schneider, T.R. Spraker, H.D. Liggitt, and D.P. Knowles. 2008. A species barrier limits transmission of chronic wasting disease to mink (Mustela vison). *Journal of General Virology* 89(Pt 4):1086-1096. https://doi.org/10.1099/vir.0.83422-0.

Haus, J.M., T.B. Eyler, M.D. Duda, and J.L. Bowman. 2017. Hunter perceptions toward chronic wasting disease: Implications for harvest and management. *Wildlife Society Bulletin* 41(2):294-300. https://doi.org/https://doi.org/10.1002/wsb.761.

Hawkins, S.A., H.A. Simmons, K.C. Gough, and B.C. Maddison. 2015. Persistence of ovine scrapie infectivity in a farm environment following cleaning and decontamination. *Veterinary Record* 176(4):99. https://doi.org/10.1136/vr.102743.

Hayward, A.D. 2022. Genetic parameters for resistance to gastrointestinal nematodes in sheep: a meta-analysis. *International Journal for Parasitology: Parasites and Wildlife* 52(13-14):843-853. https://doi.org/10.1016/j.ijpara.2022.09.004.

Head, M.W., R. Knight, M. Zeidler, H. Yull, A. Barlow, and J.W. Ironside. 2009. A case of protease sensitive prionopathy in a patient in the UK. *Neuropathology and Applied Neurobiology* 35(6):628-32. https://doi.org/10.1111/j.1365-2990.2009.01040.x.

Heberlein, T.A. 2004. "Fire in the Sistine Chapel": How Wisconsin Responded to Chronic Wasting Disease. *Human Dimensions of Wildlife*. 9 (3): 165-179. https://doi.org/10.1080/10871200490479954.

Hedman, H.D., C. Varga, J. Duquette, J. Novakofski, and N.E. Mateus-Pinilla. 2020. Food Safety Considerations Related to the Consumption and Handling of Game Meat in North America. *Veterinary Sciences* 7(4):188. https://doi.org/10.3390/vetsci7040188.

Heffelfinger, J.R., and P.R. Krausman (Eds.). 2023. Ecology and Management of Black-tailed and Mule Deer of North America. 1st ed. ed. CRC Press.

Heffelfinger, J., V. Geist, and W. Wishart. 2013. The role of hunting in North American wildlife conservation. *International Journal of Environmental Studies* 70. https://doi.org/10.1080/00207233.2013.800383.

Heggebø, R., C.M. Press, G. Gunnes, M.J. Ulvund, M.A. Tranulis, and T. Lsverk. 2003. Detection of PrPSc in lymphoid tissues of lambs experimentally exposed to the scrapie agent. *The Journal of Comparative Pathology* 128(2-3):172-81. https://doi.org/10.1053/jcpa.2002.0625.

Heisey, D.M., N.A. Mickelsen, J.R. Schneider, C.J. Johnson, C.J. Johnson, J.A. Langenberg, P.N. Bochsler, D.P. Keane, and D.J. Barr. 2010. Chronic wasting disease (CWD) susceptibility of several North American rodents that are sympatric with cervid CWD epidemics. *Journal of Virology* 84(1):210-5. https://doi.org/10.1128/jvi.00560-09.

Heikenwalder, M., N. Zeller, H. Seeger, M. Prinz, P.C. Klöhn, P. Schwarz, N.H. Ruddle, C. Weissmann, and A. Aguzzi. 2005. Chronic lymphocytic inflammation specifies the organ tropism of prions. *Science* 307(5712):1107-10. https://doi.org/10.1126/science.1106460.

Henderson, D.M., N.D. Denkers, C.E. Hoover, E.E. McNulty, S.K. Cooper, L.A. Bracchi, C.K. Mathiason, and E.A. Hoover. 2020. Progression of chronic wasting disease in white-tailed deer analyzed by serial biopsy RT-QuIC and immunohistochemistry. *PLOS ONE* 15(2):e0228327. https://doi.org/10.1371/journal.pone.0228327.

Henderson, D.M., N.D. Denkers, C.E. Hoover, N. Garbino, C.K. Mathiason, and E.A. Hoover. 2015a. Longitudinal Detection of Prion Shedding in Saliva and Urine by Chronic Wasting Disease-Infected Deer by Real-Time Quaking-Induced Conversion. *Journal of Virology* 89(18):9338-47. https://doi.org/10.1128/jvi.01118-15.

Henderson, D.M., K.A. Davenport, N.J. Haley, N.D. Denkers, C.K. Mathiason, and E.A. Hoover. 2015b. Quantitative assessment of prion infectivity in tissues and body fluids by real-time quaking-induced conversion. *Journal of General Virology* 96(1):210-219. https://doi.org/10.1099/vir.0.069906-0.

Henderson, D.M., J.M. Tennant, N.J. Haley, N.D. Denkers, C.K. Mathiason, and E.A. Hoover. 2017. Detection of chronic wasting disease prion seeding activity in deer and elk feces by real-time quaking-induced conversion. *Journal of General Virology* 98(7):1953-1962. https://doi.org/10.1099/jgv.0.000844.

Herbst, A., C.D. Velásquez, E. Triscott, J.M. Aiken, and D. McKenzie. 2017. Chronic Wasting Disease Prion Strain Emergence and Host Range Expansion. *Emerging Infectious Diseases* 23(9):1598-1600. https://doi.org/10.3201/eid2309.161474.

Herbst, A., S. Wohlgemuth, J. Yang, A.R. Castle, D.M. Moreno, A. Otero, J.M. Aiken, D. Westaway, and D. McKenzie. 2022. Susceptibility of Beavers to Chronic Wasting Disease. *Biology (Basel)* 11(5). https://doi.org/10.3390/biology11050667.

Herzog, C., N. Salès, N. Etchegaray, A. Charbonnier, S. Freire, D. Dormont, J.P. Deslys, and C.I. Lasmézas. 2004. Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. *Lancet* 363(9407):422-8. https://doi.org/10.1016/s0140-6736(04)15487-1.

Hibler, C.P., K.L. Wilson, T.R. Spraker, M.W. Miller, R.R. Zink, L.L. DeBuse, E. Andersen, D. Schweitzer, J.A. Kennedy, L.A. Baeten, J.F. Smeltzer, M.D. Salman, and B.E. Powers. 2003. Field validation and assessment of an enzyme-linked immunosorbent assay for detecting chronic wasting disease in mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus), and Rocky Mountain elk (Cervus elaphus nelsoni). *Journal of Veterinary Diagnostic Investigation* 15(4):311-9. https://doi.org/10.1177/104063870301500402.

Hildebrand, E. M. Carstensen, L. Cornicelli, D.C. Pauly, and M.H. Dexter. 2013. "Chronic Wasting Disease Management in a Minnesota Deer Herd: Coordinated Response to the Southeast Detection, 2010-2013". In *Summaries of Wildlife Research Findings 2013*, L. Cornicelli, M. Carstensen, M.D. Grund, M.A. Larson, and J.S. Lawrence editors. p. 144-154. St. Paul, MN: Minnesota Department of Natural Resources, Division of Fish and Wildlife, Wildlife Populations and Research Unit. https://files.dnr.state.mn.us/publications/wildlife/research2013/binder.pdf.

Hill, A.F., M. Desbruslais, S. Joiner, K.C. Sidle, I. Gowland, J. Collinge, L.J. Doey, and P. Lantos. 1997. The same prion strain causes vCJD and BSE. *Nature* 389(6650):448-50. https://doi.org/10.1038/38925.

Hill, A.F., and J. Collinge. 2003. Subclinical prion infection. *Trends in Microbiology* 11(12):578-584. https://doi.org/https://doi.org/10.1016/j.tim.2003.10.007.

Hill, A.F., and J. Collinge. 2004. Prion strains and species barriers. *Contributions to Microbiology* 11:33-49. https://doi.org/10.1159/000077061.

Hille, M.M., J.E. Jewell, and E.L. Belden. 2019. Cellular distribution of the prion protein in palatine tonsils of mule deer (Odocoileus hemionus) and Rocky Mountain elk (Cervus elaphus nelsoni). *Journal of Veterinary Medical Science* 81(11):1586-1596. https://doi.org/10.1292/jvms.19-0358.

Hines, A.M., V.O. Ezenwa, P. Cross, and J.D. Rogerson. 2007. Effects of supplemental feeding on gastrointestinal parasite infection in elk (Cervus elaphus): Preliminary observations. *Veterinary Parasitology*. 148 (3): 350-355. https://doi.org/10.1016/j.vetpar.2007.07.006.

Holling, C.S. 1978. *Adaptive Environmental Assessment and Management.* Laxenburg, Austria: International Institute for Applied Systems Analysis.

Holsman, R.H., J. Petchenik, and E.E. Cooney. 2010. CWD After "the Fire": Six Reasons Why Hunters Resisted Wisconsin's Eradication Effort. *Human Dimensions of Wildlife* 15(3):180-193. https://doi.org/10.1080/10871201003718029.

Hoover, C.E., K.A. Davenport, D.M. Henderson, L.A. Pulscher, C.K. Mathiason, M.D. Zabel, and E.A. Hoover. 2016. Detection and Quantification of CWD Prions in Fixed Paraffin Embedded Tissues by Real-Time Quaking-Induced Conversion. *Scientific Reports* 6(1):25098. https://doi.org/10.1038/srep25098.

Hoover, C.E., K.A. Davenport, D.M. Henderson, N.D. Denkers, C.K. Mathiason, C. Soto, M.D. Zabel, and E.A. Hoover. 2017a. Pathways of Prion Spread during Early Chronic Wasting Disease in Deer. *Journal of Virology* 91(10). https://doi.org/10.1128/jvi.00077-17.

Hoover, C.E., K.A. Davenport, D.M. Henderson, M.D. Zabel, and E.A. Hoover. 2017b. Endogenous Brain Lipids Inhibit Prion Amyloid Formation In Vitro. *Journal of Virology* 91(9). https://doi.org/10.1128/jvi.02162-16.

Huang, M.H.J., S. Demarais, A. Banda, B.K. Strickland, A.G. Welch, S. Hearst, S. Lichtenberg, A. Houston, K.M. Pepin, and K.C. VerCauteren. 2024. Expanding CWD disease surveillance options using environmental contamination at deer signposts. *Ecological Solutions and Evidence* 5(1):e12298. https://doi.org/10.1002/2688-8319.12298.

Hui, S.L., and X.H. Zhou. 1998. Evaluation of diagnostic tests without gold standards. *Statistical Methods in Medical Research* 7(4):354-370. https://doi.org/10.1177/096228029800700404.

Huillard d'Aignaux, J.N., S.N. Cousens, J. Maccario, D. Costagliola, M.P. Alpers, P.G. Smith, and A. Alpérovitch. 2002. The Incubation Period of Kuru. *Epidemiology* 13(4):402-408.

Hunter, N., J.D. Foster, W. Goldmann, M.J. Stear, J. Hope, and C. Bostock. 1996. Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. *Archives of Virology* 141(5):809-24. https://doi.org/10.1007/bf01718157.

Hunter, N. 1997. PrP genetics in sheep and the implications for scrapie and BSE. *Trends in Microbiology* 5(8):331-334. https://doi.org/https://doi.org/10.1016/S0966-842X(97)01081-0.

Huson, H.J., and G.M. Happ. 2006. Polymorphisms of the prion protein gene (PRNP) in Alaskan moose (Alces alces gigas). *Animal Genetics* 37(4):425-426. https://doi.org/10.1111/j.1365-2052.2006.01466.x.

Institute of Medicine of the National Academies, Committee on Emerging Microbial Threats to Health in the 21st Century. 2003. In *Microbial Threats to Health: Emergence, Detection, and Response*, edited by M.S. Smolinski, M.A. Hamburg and J. Lederberg. Washington (DC): National Academies Press.

Inzalaco, H.N., F. Bravo-Risi, R. Morales, D.P. Walsh, D.J. Storm, J.A. Pedersen, W.C. Turner, and S.S. Lichtenberg. 2023. Ticks harbor and excrete chronic wasting disease prions. *Scientific Reports* 13(1):7838. https://doi.org/10.1038/s41598-023-34308-3.

Inzalaco, H.N., E.E. Brandell, S.P. Wilson, M. Hunsaker, D.R. Stahler, K. Woelfel, D P. Walsh, T. Nordeen, D.J. Storm, S.S. Lichtenberg, and W.C. Turner. 2024. Detection of prions from spiked and free-ranging carnivore feces. *bioRxiv*.: 2023.07.31.551307. https://doi.org/10.1101/2023.07.31.551307.

Jacobson, K.H., S. Lee, R.A. Somerville, D. McKenzie, C.H. Benson, and J.A. Pedersen. 2010. Transport of the Pathogenic Prion Protein through Soils. *Journal of Environmental Quality* 39(4):1145-1152. https://doi.org/10.2134/jeq2009.0137.

Janousek, W.M., T.A. Graves, E.E. Berman, G.W. Chong, E.K. Cole, S.R. Dewey, A.N. Johnston, and P.C. Cross. 2021. Human activities and weather drive contact rates of wintering elk. *Journal of Applied Ecology* 58(3):667-676. https://doi.org/10.1111/1365-2664.13818.

Jennelle, C.S., M.D. Samuel, C.A. Nolden, and E.A. Berkley. 2009. Deer carcass decomposition and potential scavenger exposure to chronic wasting disease. *Journal of Wildlife Management* 73(5):655-662. https://doi.org/10.2193/2008-282.

Jennelle, C.S., D.P. Walsh, M.D. Samuel, E.E. Osnas, R. Rolley, J. Langenberg, J.G. Powers, R.J. Monello, E.D. Demarest, R. Gubler, and D.M. Heisey. 2018. Applying a Bayesian weighted surveillance approach to detect chronic wasting disease in white-tailed deer. *Journal of Applied Ecology* 55(6):2944-2953. https://doi.org/10.1111/1365-2664.13178.

Jennelle, C.S., W.D. Walter, J. Crawford, C.S. Rosenberry, and B.D. Wallingford. 2022. Movement of white-tailed deer in contrasting landscapes influences management of chronic wasting disease. *The Journal of Wildlife Management* 86(8):e22306. https://doi.org/10.1002/jwmg.22306.

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(Pt 8):2127-2134. https://doi.org/10.1099/vir.0.81077-0.

Jewell, J.E., J. Brown, T. Kreeger, and E.S. Williams. 2006. Prion protein in cardiac muscle of elk (Cervus elaphus nelsoni) and white-tailed deer (Odocoileus virginianus) infected with chronic wasting disease. *Journal of General Virology* 87(11):3443-3450. https://doi.org/https://doi.org/10.1099/vir.0.81777-0.

Johnson, C., J. Johnson, M. Clayton, D. McKenzie, and J. Aiken. 2003. Prion protein gene heterogeneity in free-ranging white-tailed deer within the chronic wasting disease affected region of Wisconsin. *Journal of Wildlife Diseases* 39(3):576-81. https://doi.org/10.7589/0090-3558-39.3.576.

Johnson, C., J. Johnson, J.P. Vanderloo, D. Keane, J.M. Aiken, and D. McKenzie. 2006a. Prion protein polymorphisms in white-tailed deer influence susceptibility to chronic wasting disease. *Journal of General Virology* 87(Pt 7):2109-2114. https://doi.org/10.1099/vir.0.81615-0.

Johnson, C.J., K.E. Phillips, P.T. Schramm, D. McKenzie, J.M. Aiken, and J.A. Pedersen. 2006b. Prions Adhere to Soil Minerals and Remain Infectious. *PLOS Pathogens* 2(4):e32. https://doi.org/10.1371/journal.ppat.0020032.

Johnson, C.J., J.A. Pedersen, R.J. Chappell, D. McKenzie, and J.M. Aiken. 2007. Oral Transmissibility of Prion Disease Is Enhanced by Binding to Soil Particles. *PLOS Pathogens* 3(7):e93. https://doi.org/10.1371/journal.ppat.0030093.

Johnson, C.J., A. Herbst, C. Duque-Velasquez, J.P. Vanderloo, P. Bochsler, R. Chappell, and D. McKenzie. 2011a. Prion Protein Polymorphisms Affect Chronic Wasting Disease Progression. *PLOS ONE* 6(3):e17450. https://doi.org/10.1371/journal.pone.0017450.

Johnson, C.J., D. McKenzie, J.A. Pedersen, and J.M. Aiken. 2011b. Meat and bone meal and mineral feed additives may increase the risk of oral prion disease transmission. *Journal of Toxicology and Environmental Health* (74)Part A(2-4):161-6. https://doi.org/10.1080/15287394.2011.529066.

Joly, D.O., M.D. Samuel, J.A. Langenberg, R.E. Rolley, and D.P. Keane. 2009. Surveillance to detect chronic wasting disease in white-tailed deer in Wisconsin. *Journal of Wildlife Diseases* 45(4):989-97. https://doi.org/10.7589/0090-3558-45.4.989.

Jones, R.M., M. Nicas, A. Hubbard, M.D. Sylvester, and A. Reingold. 2005. The Infectious Dose of Francisella Tularensis (Tularemia). *Applied Biosafety* 10(4):227-239. https://doi.org/10.1177/153567600501000405.

Kaatz, M., C. Fast, U. Ziegler, A. Balkema-Buschmann, B. Hammerschmidt, M. Keller, A. Oelschlegel, L. McIntyre, and M.H. Groschup. 2012. Spread of Classic BSE Prions from the Gut via the Peripheral Nervous System to the Brain. *The American Journal of Pathology* 181(2):515-524. https://doi.org/10.1016/j.ajpath.2012.05.001.

Kahn, S., C. Dubé, L. Bates, and A. Balachandran. 2004. Chronic wasting disease in Canada: Part 1. *Canadian Veterinary Journal* 45(5):397-404.

Kim, T.Y., H.J. Shon, Y.S. Joo, U.K. Mun, K.S. Kang, and Y.S. Lee. 2005. Additional Cases of Chronic Wasting Disease in Imported Deer in Korea. *Journal of Veterinary Medical Science* 67(8):753-759. https://doi.org/10.1292/jvms.67.753.

Kjær, L.J., E.M. Schauber, and C.K. Nielsen. 2008. Spatial and Temporal Analysis of Contact Rates in Female White-Tailed Deer. *The Journal of Wildlife Management* 72(8):1819-1825. https://doi.org/10.2193/2007-489.

Kjær, L.J., and E.M. Schauber. 2022. The effect of landscape, transmission mode and social behavior on disease transmission: Simulating the transmission of chronic wasting disease in white-tailed deer (Odocoileus virginianus) populations using a spatially explicit agent-based model. *Ecological Modelling* 472:110114. https://doi.org/10.1016/j.ecolmodel.2022.110114.

Kaiser-Schulz, G., A. Heit, L. Quintanilla-Martinez, F. Hammerschmidt, S. Hess, L. Jennen, H. Rezaei, H. Wagner, and H.M. Schätzl. 2007. Polylactide-coglycolide microspheres co-encapsulating recombinant tandem prion protein with CpG-oligonucleotide break self-tolerance to prion protein in wild-type mice and induce CD4 and CD8 T cell responses. *Journal of Immunology* 179(5):2797-807. https://doi.org/10.4049/jimmunol.179.5.2797.

Kamali-Jamil, R., E. Vázquez-Fernández, B. Tancowny, V. Rathod, S. Amidian, X. Wang, X. Tang, A. Fang, A. Senatore, S. Hornemann, S. Dudas, A. Aguzzi, H.S. Young, and H. Wille. 2021. The ultrastructure of infectious L-type bovine spongiform encephalopathy prions constrains molecular models. *PLOS Pathogens* 17(6):e1009628. https://doi.org/10.1371/journal.ppat.1009628.

Kascsak, R.J., Rubenstein, R., Carp, R.I. 1991. Evidence for Biological and Structural Diversity Among Scrapie Strains. In: Chesebro, B.W. (eds) Transmissible Spongiform Encephalopathies: Current Topics in Microbiology and Immunology, Vol 172. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-76540-7_9.

Keane, D.P., D.J. Barr, P.N. Bochsler, S.M. Hall, T. Gidlewski, K.I. O'Rourke, T.R. Spraker, and M.D. Samuel. 2008a. Chronic Wasting Disease in a Wisconsin White-Tailed Deer Farm. *Journal of Veterinary Diagnostic Investigation* 20(5):698-703. https://doi.org/10.1177/104063870802000534.

Keane, D.P., D.J. Barr, J.E. Keller, S.M. Hall, J.A. Langenberg, and P.N. Bochsler. 2008b. Comparison of retropharyngeal lymph node and obex region of the brainstem in detection of chronic wasting disease in white-tailed deer (Odocoileus virginianus). *Journal of Veterinary Diagnostic Investigation* 20(1):58-60. https://doi.org/10.1177/104063870802000110.

Keane, D., D. Barr, R. Osborn, J. Langenberg, K. O'Rourke, D. Schneider, and P. Bochsler. 2009. Validation of use of rectoanal mucosa-associated lymphoid tissue for immunohistochemical diagnosis of chronic wasting disease in white-tailed deer (Odocoileus virginianus). *Journal of Clinical Microbiology* 47(5):1412-7. https://doi.org/10.1128/jcm.02209-08.

Kelly, A.C., N.E. Mateus-Pinilla, J. Diffendorfer, E. Jewell, M.O. Ruiz, J. Killefer, P. Shelton, T. Beissel, and J. Novakofski. 2008. Prion sequence polymorphisms and chronic wasting disease resistance in Illinois white-tailed deer (Odocoileus virginianus). *Prion* 2(1):28-36. https://doi.org/10.4161/pri.2.1.6321.

Ketz, A.C., D.J. Storm, and M. Samuel. 2019. Chronic wasting disease and implications for cervid populations. *CABI Reviews* 1–15. https://doi.org/10.1079/pavsnnr201914038.

Ketz, A.C., S.J. Robinson, C.J. Johnson, and M.D. Samuel. 2022. Pathogen-mediated selection and management implications for white-tailed deer exposed to chronic wasting disease. *Journal of Applied Ecology* 59(4):982-996. https://doi.org/https://doi.org/10.1111/1365-2664.14109.

Kincheloe, J.M., A.R. Horn-Delzer, D.N. Makau, and S.J. Wells. 2021. Chronic Wasting Disease Transmission Risk Assessment for Farmed Cervids in Minnesota and Wisconsin. *Viruses* 13(8). https://doi.org/10.3390/v13081586.

Kimberlin, Richard H., Carol A. Walker, and Hugh Fraser. 1989. The Genomic Identity of Different Strains of Mouse Scrapie Is Expressed in Hamsters and Preserved on Reisolation in Mice. *Journal of General Virology* 70(8):2017-2025. https://doi.org/10.1099/0022-1317-70-8-2017.

Kirkwood, J.K., and A.A. Cunningham. 1994. Epidemiological observations on spongiform encephalopathies in captive wild animals in the British Isles. *Veterinary Record* 135(13):296-303. https://doi.org/10.1136/vr.135.13.296.

Klingeborn, M., L. Wik, M. Simonsson, L.H.M. Renström, T. Ottinger, and T. Linné. 2006. Characterization of proteinase K-resistant N- and C-terminally truncated PrP in Nor98 atypical scrapie. Journal of General Virology. 87 (Pt 6): 1751-1760. https://doi.org/10.1099/vir.0.81618-0.

Koets, A.P., G. Adugna, L.L.G. Janss, H.J. van Weering, C.H.J. Kalis, G.H. Wentink, V.P.M.G. Rutten, and Y.H. Schukken. 2000. Genetic Variation of Susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* Infection in Dairy Cattle. *Journal of Dairy Science* 83(11):2702-2708. https://doi.org/10.3168/jds.S0022-0302(00)75164-2.

Kong, Q., S. Huang, W. Zou, D. Vanegas, M. Wang, D. Wu, J. Yuan, M. Zheng, H. Bai, H. Deng, K. Chen, A.L. Jenny, K. O'Rourke, E.D. Belay, L.B. Schonberger, R.B. Petersen, M.-S. Sy, S.G. Chen, and P. Gambetti. 2005. Chronic Wasting Disease of Elk: Transmissibility to Humans Examined by Transgenic Mouse Models. *The Journal of Neuroscience* 25(35):7944-7949. https://doi.org/10.1523/jneurosci.2467-05.2005.

Kramm, C., R. Gomez-Gutierrez, C. Soto, G. Telling, T. Nichols, and R. Morales. 2019. In Vitro detection of Chronic Wasting Disease (CWD) prions in semen and reproductive tissues of white-tailed deer bucks (Odocoileus virginianus). *PLOS ONE* 14(12):e0226560. https://doi.org/10.1371/journal.pone.0226560.

Kramm, C., S. Pritzkow, A. Lyon, T. Nichols, R. Morales, and C. Soto. 2017. Detection of Prions in Blood of Cervids at the Asymptomatic Stage of Chronic Wasting Disease. *Scientific Reports* 7(1):17241. https://doi.org/10.1038/s41598-017-17090-x.

Kraus, A., F. Hoyt, C.L. Schwartz, B. Hansen, E. Artikis, A.G. Hughson, G.J. Raymond, B. Race, G.S. Baron, and B. Caughey. 2021. High-resolution structure and strain comparison of infectious mammalian prions. *Molecular Cell* 81(21):4540-4551. https://doi.org/10.1016/j.molcel.2021.08.011.

Krausman, P., S. Christensen, J. McDonald, and B. Leopold. 2014. Dynamics and social issues of overpopulated deer ranges in the United States: a long-term assessment. *California Fish and Game* 100:436-450.

Kreeger, T.J., D.L. Montgomery, J.E. Jewell, W. Schultz, and E.S. Williams. 2006. Oral transmission of chronic wasting disease in captive Shira's moose. *Journal of Wildlife Diseases* 42(3):640-5. https://doi.org/10.7589/0090-3558-42.3.640.

Krumm, C.E., M.M. Conner, and M.W. Miller. 2005. Relative vulnerability of chronic wasting disease infected mule deer to vehicle collisions. *Journal of Wildlife Diseases* 41(3):503-11. https://doi.org/10.7589/0090-3558-41.3.503.

Krumm, C.E., M.M. Conner, N.T. Hobbs, D.O. Hunter, and M.W. Miller. 2010. Mountain lions prey selectively on prion-infected mule deer. *Biology Letters* 6(2):209-11. https://doi.org/10.1098/rsbl.2009.0742.

Kujala, P., C.R. Raymond, M. Romeijn, S.F. Godsave, S.I. van Kasteren, H. Wille, S.B. Prusiner, N.A. Mabbott, and P.J. Peters. 2011. Prion uptake in the gut: identification of the first uptake and replication sites. *PLOS Pathogens* 7(12):e1002449. https://doi.org/10.1371/journal.ppat.1002449.

Kurt, T.D., M.R. Perrott, C.J. Wilusz, J. Wilusz, S. Supattapone, G.C. Telling, M.D. Zabel, and E.A. Hoover. 2007. Efficient in vitro amplification of chronic wasting disease PrPRES. *Journal of Virology* 81(17):9605-8. https://doi.org/10.1128/jvi.00635-07.

Kuznetsova, A., C. Cullingham, D. McKenzie, and J.M. Aiken. 2018. Soil humic acids degrade CWD prions and reduce infectivity. *PLOS Pathogens* 14(11):e1007414. https://doi.org/10.1371/journal.ppat.1007414.

Kuznetsova, A., D. McKenzie, C. Cullingham, and J.M. Aiken. 2020. Long-Term Incubation PrP^{CWD} with Soils Affects Prion Recovery but Not Infectivity. *Pathogens* 9(4):311. https://doi.org/10.3390/pathogens9040311.

Kuznetsova, A., D. McKenzie, B. Ytrehus, K.S. Utaaker, and J.M. Aiken. 2023. Movement of Chronic Wasting Disease Prions in Prairie, Boreal and Alpine Soils. *Pathogens* 12(2):269. https://doi.org/10.3390/pathogens12020269.

Kuznetsova, A., A. Ness, E. Moffatt, T. Bollinger, D. McKenzie, I. Stasiak, C.S. Bahnson, and J.M. Aiken. 2024. Detection of Chronic Wasting Disease Prions in Prairie Soils from Endemic Regions. *Environmental Science & Technology* 58(25):10932-10940. https://doi.org/10.1021/acs.est.4c04633.

Kwan, P., H. Konno, K. Y. Chan, and L. Baum. 2020. Rationale for the development of an Alzheimer's disease vaccine. *Human Vaccines & Immunotherapeutics* 16(3):645-653. https://doi.org/10.1080/21645515.2019.1665453.

LaBarge, L.R., M.J. Evans, J.R.B. Miller, G. Cannataro, C. Hunt, and L.M. Elbroch. 2022. Pumas Puma concolor as ecological brokers: a review of their biotic relationships. *Mammal Review* 52(3):360-376. https://doi.org/10.1111/mam.12281.

LaCava, M.E.F., J.L. Malmberg, W.H. Edwards, L.N.L. Johnson, S.E. Allen, and H.B. Ernest. 2021. Spatio-temporal analyses reveal infectious disease-driven selection in a free-ranging ungulate. *Royal Society Open Science* 8(8):210802. https://doi.org/10.1098/rsos.210802.

Lacroux, C., H. Cassard, H. Simmons, J.Y. Douet, F. Corbière, S. Lugan, P. Costes, N. Aron, A. Huor, C. Tillier, F. Schelcher, and Ol Andreoletti. Classical scrapie transmission in ARR/ARR genotype sheep. *Journal of General Virology* 98(8). https://doi.org/10.1099/jgv.0.000861.

LaFauci, G., R.I. Carp, H.C. Meeker, X. Ye, J.I. Kim, M. Natelli, M. Cedeno, R.B. Petersen, R. Kascsak, and R. Rubenstein. 2006. Passage of chronic wasting disease prion into transgenic mice expressing Rocky Mountain elk (Cervus elaphus nelsoni) PrPC. *Journal of General Virology* 87(Pt 12):3773-3780. https://doi.org/10.1099/vir.0.82137-0.

Landon, A.C., D.C. Fulton, A.K. Pradhananga, L. Cornicelli, and M.A. Davenport. 2021. Community Attachment and Stewardship Identity Influence Responsibility to Manage Wildlife. *Society & Natural Resources* 34(5):571-584. https://doi.org/10.1080/08941920.2020.1852636.

Landon, A.C., K. Smith, L. Cornicelli, D.C. Fulton, L.E. McInenly, and S.A. Schroeder. Examining landowners' preferences for a chronic wasting disease management program. *Wildlife Society Bulletin* 47(1):e1401. https://doi.org/10.1002/wsb.1401.

Lasmézas, C.I., J.P. Deslys, O. Robain, A. Jaegly, V. Beringue, J.M. Peyrin, J.G. Fournier, J.J. Hauw, J. Rossier, and D. Dormont. 1997. Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein. *Science* 275(5298):402-5. https://doi.org/10.1126/science.275.5298.402.

Laurenson, I.F., A.S. Whyte, and C. Fox. 2001. Iatrogenic Prion Infection (Letter to the Editor). *The New England Journal of Medicine* 345(11):840-841. https://doi.org/10.1056/NEJM200109133451116.

Leong, K., and D.J. Decker. 2020. Human dimensions considerations in wildlife disease management. Reston, VA: U.S. Geological Survey.

Levavasseur, E., A.-G. Biacabe, E. Comoy, A. Culeux, K. Grznarova, N. Privat, S. Simoneau, B. Flan, V. Sazdovitch, D. Seilhean, T. Baron, and S. Haïk. 2017. Detection and partial discrimination of atypical and classical bovine spongiform encephalopathies in cattle and primates using real-time quaking-induced conversion assay. *PLOS ONE* 12(2):e0172428. https://doi.org/10.1371/journal.pone.0172428.

Li, M., M.D. Schwabenlander, G.R. Rowden, J.M. Schefers, C.S. Jennelle, M. Carstensen, D. Seelig, and P.A. Larsen. 2021. RT-QuIC detection of CWD prion seeding activity in white-tailed deer muscle tissues. *Scientific Reports* 11(1):16759. https://doi.org/10.1038/s41598-021-96127-8.

Lichtenberg, S.S., S. Thomas, D. Storm, D. Walsh, M. Milstein, S. Gretsch, M. Schwabenlander, and T. Wolf. 2023. Recent advances in environmental prion detection and remediation. 266th American Chemical Society National Meeting.

Ligios, C., C.J. Sigurdson, C. Santucciu, G. Carcassola, G. Manco, M. Basagni, C. Maestrale, M.G. Cancedda, L. Madau, and A. Aguzzi. 2005. PrPSc in mammary glands of sheep affected by scrapie and mastitis. *Nature Medicine* 11(11):1137-1138. https://doi.org/10.1038/nm1105-1137.

Lischka, S.A., P. Shelton, and J. Buhnerkempe. 2010. Support for Chronic Wasting Disease Management Among Residents of the Infected Area in Illinois. *Human Dimensions of Wildlife* 15(3):229-232. https://doi.org/10.1080/10871201003736054.

Maddox, R.A., M.K. Person, J.E. Blevins, J.Y. Abrams, B.S. Appleby, L.B. Schonberger, and E.D. Belay. 2020. Prion disease incidence in the United States: 2003-2015. *Neurology* 94(2):e153-e157. https://doi.org/10.1212/wnl.0000000000008680.

Makarava, Natallia, Tarek Safadi, Olga Bocharova, Olga Mychko, Narayan P. Pandit, Kara Molesworth, Simone Baiardi, Li Zhang, Piero Parchi, and Ilia V. Baskakov. 2024. Reactive microglia partially envelop viable neurons in prion diseases. *The Journal of Clinical Investigation*. https://doi.org/10.1172/JCI181169.a.

Makau, D.N., K. VanderWaal, J. Kincheloe, and S.J. Wells. 2020. Implications of farmed-cervid movements on the transmission of chronic wasting disease. *Preventive Veterinary Medicine* 182:105088. https://doi.org/10.1016/j.prevetmed.2020.105088.

Mallikarjun, A., B. Swartz, S.A. Kane, M. Gibison, I. Wilson, A. Collins, M.B. Moore, I. Charendoff, J. Ellis, L.A. Murphy, T. Nichols, and C.M. Otto. 2023. Canine detection of chronic wasting disease (CWD) in laboratory and field settings. *Prion* 17(1):16-28. https://doi.org/10.1080/19336896.2023.2169519.

Mammadova, N., E. Cassmann, and J.J. Greenlee. 2020. Efficient transmission of classical scrapie agent x124 by intralingual route to genetically susceptible sheep with a low dose inoculum. *Research in Veterinary Science* 132:217-220. https://doi.org/10.1016/j.rvsc.2020.06.010.

Manjerovic, M.B., M.L. Green, N. Mateus-Pinilla, and J. Novakofski. 2014. The importance of localized culling in stabilizing chronic wasting disease prevalence in white-tailed deer populations. *Preventive Veterinary Medicine* 113(1):139-145. https://doi.org/10.1016/j.prevetmed.2013.09.011.

Manka, Szymon W., Adam Wenborn, John Collinge, and Jonathan D. F. Wadsworth. 2023a. Prion strains viewed through the lens of cryo-EM. *Cell and Tissue Research* 392(1):167-178. https://doi.org/10.1007/s00441-022-03676-z.

Manka, Szymon W., Adam Wenborn, Jemma Betts, Susan Joiner, Helen R. Saibil, John Collinge, and Jonathan D. F. Wadsworth. 2023b. A structural basis for prion strain diversity. *Nature Chemical Biology* 19(5):607-613. https://doi.org/10.1038/s41589-022-01229-7.

Manne, S., N. Kondru, T. Nichols, A. Lehmkuhl, B. Thomsen, R. Main, P. Halbur, S. Dutta, and A.G. Kanthasamy. 2017. Ante-mortem detection of chronic wasting disease in recto-anal mucosa-associated lymphoid tissues from elk (Cervus elaphus nelsoni) using real-time quaking-induced conversion (RT-QuIC) assay: A blinded collaborative study. *Prion* 11(6):415-430. https://doi.org/10.1080/19336896.2017.1368936.

Marsh, R.F., A.E. Kincaid, R.A. Bessen, and J.C. Bartz. 2005. Interspecies transmission of chronic wasting disease prions to squirrel monkeys (*Saimiri sciureus*). *Journal of Virology* 79(21):13794-6. https://doi.org/10.1128/jvi.79.21.13794-13796.2005.

Martin, S.W., A.H. Meek, and P. Willeberg. 1987. *Veterinary Epidemiology: Principles and Methods*. 1st ed. Ames, IA: Iowa State University Press.

Maraud, S., and S. Roturier. 2021. Chronic Wasting Disease (CWD) in Sami Reindeer Herding: The Socio-Political Dimension of an Epizootic in an Indigenous Context. *Animals (Basel)* 11(2). https://doi.org/10.3390/ani11020297.

Masujin, K., C.D. Orrú, K. Miyazawa, B.R. Groveman, L.D. Raymond, A.G. Hughson, and B. Caughey. 2016. Detection of Atypical H-Type Bovine Spongiform Encephalopathy and Discrimination of Bovine Prion Strains by Real-Time Quaking-Induced Conversion. *Journal of Clinical Microbiology* 54(3):676-686. https://doi.org/doi:10.1128/jcm.02731-15.

Mathiason, C.K., J.G. Powers, S.J. Dahmes, D.A. Osborn, K.V. Miller, R.J. Warren, G.L. Mason, S.A. Hays, J. Hayes-Klug, D.M. Seelig, M.A. Wild, L.L. Wolfe, T.R. Spraker, M.W. Miller, C.J. Sigurdson, G.C. Telling, and E.A. Hoover. 2006. Infectious Prions in the Saliva and Blood of Deer with Chronic Wasting Disease. *Science* 314(5796):133-136. https://doi.org/doi:10.1126/science.1132661.

Mathiason, C.K., S.A. Hays, J. Powers, J. Hayes-Klug, J. Langenberg, S.J. Dahmes, D.A. Osborn, K.V. Miller, R.J. Warren, G.L. Mason, and E.A. Hoover. 2009. Infectious Prions in Pre-Clinical Deer and Transmission of Chronic Wasting Disease Solely by Environmental Exposure. *PLOS ONE* 4(6):e5916. https://doi.org/10.1371/journal.pone.0005916.

Mathiason, C.K., J. Hayes-Klug, S.A. Hays, J. Powers, D.A. Osborn, S.J. Dahmes, K.V. Miller, R.J. Warren, G.L. Mason, G.C. Telling, A.J. Young, and E.A. Hoover. 2010. B cells and platelets harbor prion infectivity in the blood of deer infected with chronic wasting disease. *Journal of Virology* 84(10):5097-107. https://doi.org/10.1128/jvi.02169-09.

Mathiason, C.K., A.V. Nalls, D.M. Seelig, S.L. Kraft, K. Carnes, K.R. Anderson, J. Hayes-Klug, and E.A. Hoover. 2013. Susceptibility of domestic cats to chronic wasting disease. *Journal of Virology* 87(4):1947-56. https://doi.org/10.1128/jvi.02592-12.

Mattson, J.W. and W.W. Koo. 2007. Effects of Bovine Spongiform Encephalopathy Outbreaks on U.S. Cattle and Beef Prices. *Review of Agricultural Economics* 29(4):734-748.

Mawhinney, S., W.J. Pape, J.E. Forster, C.A. Anderson, P. Bosque, and M.W. Miller. 2006. Human prion disease and relative risk associated with chronic wasting disease. *Emerging Infectious Diseases* 12(10):1527-35. https://doi.org/10.3201/eid1210.060019.

McArthur, D. White Earth Nation. December 11, 2023.

McBride, P.A., W.J. Schulz-Schaeffer, M. Donaldson, M. Bruce, H. Diringer, H.A. Kretzschmar, and M. Beekes. 2001. Early spread of scrapie from the gastrointestinal tract to the central nervous system involves autonomic fibers of the splanchnic and vagus nerves. *Journal of Virology* 75(19):9320-7. https://doi.org/10.1128/jvi.75.19.9320-9327.2001.

McLean, A.R., and C.J. Bostock. 2000. Scrapie infections initiated at varying doses: an analysis of 117 titration experiments. *Philosophical Transactions of the Royal Society B: Biological Sciences* 355(1400):1043-50. https://doi.org/10.1098/rstb.2000.0641.

McNulty, E., A.V. Nalls, S. Mellentine, E. Hughes, L. Pulscher, E.A. Hoover, and C.K. Mathiason. 2019. Comparison of conventional, amplification and bio-assay detection methods for a chronic wasting disease inoculum pool. *PLOS ONE* 14(5):e0216621. https://doi.org/10.1371/journal.pone.0216621.

Meeks, A., N.C. Poudyal, L.I. Muller, and C. Yoest. 2022. Hunter acceptability of chronic wasting disease (CWD) management actions in Western Tennessee. *Human Dimensions of Wildlife* 27(5):457-471. https://doi.org/10.1080/10871209.2021.1959962.

Mejía-Salazar, M.F., C.L. Waldner, Y.T. Hwang, and T.K. Bollinger. 2018. Use of environmental sites by mule deer: a proxy for relative risk of chronic wasting disease exposure and transmission. *Ecosphere*. 9 (1): e02055. https://doi.org/10.1002/ecs2.2055.

Melchior, M.B., J.J. Windig, T.J. Hagenaars, A. Bossers, A. Davidse, and F.G. van Zijderveld. 2010. Eradication of scrapie with selective breeding: are we nearly there? *BMC Veterinary Research* 6(1):24. https://doi.org/10.1186/1746-6148-6-24.

Menard, J., K. Jensen, and B.C. English. 2004. Projected economic impacts of a chronic wasting disease (CWD) outbreak in Tennessee. Agri-Industry Modeling & Analysis Group Industry Brief.

Merchant, R.M., E.C. South, and N. Lurie. 2021. Public Health Messaging in an Era of Social Media. *Journal of the American Medical Association* 325(3):223-224. https://doi.org/10.1001/jama.2020.24514.

Migliore, S., R. Puleio, and G.R. Loria. 2020. Scrapie Control in EU Goat Population: Has the Last Gap Been Overcome? *Frontiers in Veterinary Science* 7:581969. https://doi.org/10.3389/fvets.2020.581969.

Miller, C.A. 2003. Hunter Perceptions and Behaviors Related to Chronic Wasting Disease in Northern Illinois. *Human Dimensions of Wildlife* 8:229-230. https://doi.org/10.1080/10871200390215669.

Miller, C.A. 2004. Deer Hunter Participation and Chronic Wasting Disease in Illinois: An Assessment at Time Zero. *Human Dimensions of Wildlife* 9(3):237-239. https://doi.org/10.1080/10871200490480033.

Miller, M.W., M.A. Wild, and E.S. Williams. 1998. Epidemiology of chronic wasting disease in captive Rocky Mountain elk. *Journal of Wildlife Diseases* 34(3):532-8. https://doi.org/10.7589/0090-3558-34.3.532.

Miller, M.W., E.S. Williams, C.W. McCarty, T.R. Spraker, T.J. Kreeger, C.T. Larsen, and E.T. Thorne. 2000. Epizootiology of chronic wasting disease in free-ranging cervids in Colorado and Wyoming. *Journal of Wildlife Diseases* 36(4):676-90. https://doi.org/10.7589/0090-3558-36.4.676.

Miller, M.W., and E.S. Williams. 2002. Detection of Prp^{CWD} in mule deer by immunohistochemistry of lymphoid tissues. *Veterinary Record* 151(20):610-612. https://doi.org/10.1136/vr.151.20.610.

Miller, M.W., and E.S. Williams. 2003. Horizontal prion transmission in mule deer. *Nature* 425(6953):35-36. https://doi.org/10.1038/425035a.

Miller, M.W., E.S. Williams, N.T. Hobbs, and L.L. Wolfe. 2004a. Environmental sources of prion transmission in mule deer. *Emerging Infectious Diseases* 10(6):1003-6. https://doi.org/10.3201/eid1006.040010.

Miller, M.W., and M.A. Wild. 2004b. Epidemiology of chronic wasting disease in captive white-tailed and mule deer. *Journal of Wildlife Diseases* 40(2):320-7. https://doi.org/10.7589/0090-3558-40.2.320.

Miller, M.W., N.T. Hobbs, and S.J. Tavener. 2006. Dynamics of prion disease transmission in mule deer. *Journal of Applied Ecology* 16(6):2208-14. https://doi.org/10.1890/1051-0761.

Miller, M.W., H.M. Swanson, L.L. Wolfe, F.G. Quartarone, S.L. Huwer, C.H. Southwick, and P.M. Lukacs. 2008. Lions and Prions and Deer Demise. *PLOS ONE* 3(12):e4019. https://doi.org/10.1371/journal.pone.0004019.

Miller, M.W., L.L. Wolfe, T.M. Sirochman, M.A. Sirochman, J.E. Jewell, and E.S. Williams. 2012. Survival Patterns in White-tailed and Mule Deer after Oral Inoculation with a Standardized, Conspecific Prion Dose. *Journal of Wildlife Diseases* 48(2):526-529. https://doi.org/10.7589/0090-3558-48.2.526.

Miller, J.E., and D.A. Miller. 2016. Introduction: Ecological, biological, economic, and social issues associated with captive cervids. *Wildlife Society Bulletin* 40(1):7-9. https://doi.org/https://doi.org/10.1002/wsb.639.

Miller, M.W., and J.R. Fischer. 2016. The first five (or more) decades of chronic wasting disease: lessons for the five decades to come. Transactions of the North American Wildlife and Natural Resources Conference.

Miller, M.W., J.P. Runge, A. A. Holland, and M.D. Eckert. 2020. Hunting Pressure Modulates Prion Infection Risk in Mule Deer Herds. *Journal of Wildlife Diseases* 56(4):781-790. https://doi.org/10.7589/jwd-d-20-00054.

Miller, M.W., and L.L. Wolfe. 2021. Inferring Chronic Wasting Disease Incidence from Prevalence Data. *Journal of Wildlife Diseases* 57(3):718-721. https://doi.org/10.7589/jwd-d-20-00216.

Miller, M.W., and L.L. Wolfe. 2023. Chronic Wasting Disease. Edited by Robin W. Radcliffe, and David A. Jessup (eds). Wildlife Disease and Health in Conservation Johns Hopkins University Press.

Miller, M.W. and E.S. Williams. 2001. Chronic Wasting Disease of Deer and Elk. USAHA Newsletter: USAHA.

Millhauser, G. L. 2004. Copper Binding in the Prion Protein. *Accounts of Chemical Research* 37(2):79-85. https://doi.org/10.1021/ar0301678.

Milstein, Marissa, Sarah C. Gresch, Marc D. Schwabenlander, Manci Li, Jason C. Bartz, Damani N. Bryant, Peter R. Christenson, Laramie L. Lindsey, Nicole Lurndahl, Sang-Hyun Oh, Gage R. Rowden, Rachel L. Shoemaker, Tiffany M. Wolf, Peter A. Larsen, and Stuart S. Lichtenberg. 2024. Detection and decontamination of chronic wasting disease prions during venison processing. *bioRxiv*. https://doi.org/10.1101/2024.07.23.604851.

Minnesota Department of Natural Resources. 2019. Surveillance and Management Plan for Chronic Wasting Disease in Free-ranging Cervids in Minnesota (updated July 2024). https://files.dnr.state.mn.us/wildlife/research/health/disease/cwd/cwd_responseplan.pdf.

Minich, D., C. Madden, M.V. Evans, G.A. Ballash, D.J. Barr, K.P. Poulsen, P.M. Dennis, and V.L. Hale. 2021. Alterations in gut microbiota linked to provenance, sex, and chronic wasting disease in white-tailed deer (Odocoileus virginianus). *Scientific Reports* 11(1):13218. https://doi.org/10.1038/s41598-021-89896-9.

Mitchell, G.B., C.J. Sigurdson, K.I. O'Rourke, J. Algire, N.P. Harrington, I. Walther, T.R. Spraker, and A. Balachandran. 2012. Experimental Oral Transmission of Chronic Wasting Disease to Reindeer (Rangifer tarandus tarandus). *PLOS ONE* 7(6):e39055. https://doi.org/10.1371/journal.pone.0039055.

Mitchell, G., N. Yogasingam, I. Walther, and A. Balachandran. 2015. Experimental transmission of chronic wasting disease to sheep and goats. *Prion*. Taylor and Francis.

Monello, R.J., J.G. Powers, N.T. Hobbs, T.R. Spraker, M.K. Watry, and M.A. Wild. 2014. Survival and population growth of a free-ranging elk population with a long history of exposure to chronic wasting disease. *The Journal of Wildlife Management* 78(2):214-223. https://doi.org/https://doi.org/10.1002/jwmg.665.

Monello, R.J., N.L. Galloway, J.G. Powers, S.A. Madsen-Bouterse, W.H. Edwards, M.E. Wood, K.I. O'Rourke, and M.A. Wild. 2017. Pathogen-mediated selection in free-ranging elk populations infected by chronic wasting disease. *Proceedings of the National Academy of Sciences* 114(46):12208-12212. https://doi.org/doi:10.1073/pnas.1707807114.

Montrasio, F., R. Frigg, M. Glatzel, M.A. Klein, F. Mackay, A. Aguzzi, and C. Weissmann. 2000. Impaired prion replication in spleens of mice lacking functional follicular dendritic cells. *Science* 288(5469):1257-9. https://doi.org/10.1126/science.288.5469.1257.

Moore, S.J., R. Kunkle, M.H. Greenlee, E. Nicholson, J. Richt, A. Hamir, W.R. Waters, and J. Greenlee. 2016. Horizontal Transmission of Chronic Wasting Disease in Reindeer. *Emerging Infectious Diseases* 22(12):2142-2145. https://doi.org/10.3201/eid2212.160635.

Moore, S.J., M.H. West Greenlee, N. Kondru, S. Manne, J.D. Smith, R.A. Kunkle, A. Kanthasamy, and J.J. Greenlee. 2017. Experimental Transmission of the Chronic Wasting Disease Agent to Swine after Oral or Intracranial Inoculation. *Journal of Virology* 91(19). https://doi.org/doi:10.1128/jvi.00926-17.

Moore, S.J., C.E. Vrentas, S. Hwang, M.H. West Greenlee, E.M. Nicholson, and J.J. Greenlee. 2018. Pathologic and biochemical characterization of PrPSc from elk with PRNP polymorphisms at codon 132 after experimental infection with the chronic wasting disease agent. *BMC Veterinary Research* 14(1):80. https://doi.org/10.1186/s12917-018-1400-9.

Moore, S.J., J.D. Smith, J.A. Richt, and J.J. Greenlee. 2019. Raccoons accumulate PrP(Sc) after intracranial inoculation of the agents of chronic wasting disease or transmissible mink encephalopathy but not atypical scrapie. *Journal of Veterinary Diagnostic Investigation* 31(2):200-209. https://doi.org/10.1177/1040638718825290.

Moore, J., T. Tatum, S. Hwang, C. Vrentas, M.H. West Greenlee, Q. Kong, E. Nicholson, and J. Greenlee. 2020. Novel Strain of the Chronic Wasting Disease Agent Isolated from Experimentally Inoculated Elk with LL132 Prion Protein. *Scientific Reports* 10(1):3148. https://doi.org/10.1038/s41598-020-59819-1.

Moore, S., W. Severud, T. Wolf, K. Pelican, J. Bauerkemper, M. Carstensen, and S. Windels. 2024. Indigenous co-stewardship of North American moose: recommendations and a vision for a restoration framework. *Journal of Wildlife Management* 88. https://doi.org/10.1002/jwmg.22623.

Morales, Rodrigo. 2017. Prion strains in mammals: Different conformations leading to disease. *PLOS Pathogens* 13(7):e1006323. https://doi.org/10.1371/journal.ppat.1006323.

Morales, R., C. Duran-Aniotz, R. Diaz-Espinoza, M.V. Camacho, and C. Soto. 2012. Protein misfolding cyclic amplification of infectious prions. *Nature Protocols* 7(7):1397-1409. https://doi.org/10.1038/nprot.2012.067.

Morales, R. 2017. Prion strains in mammals: Different conformations leading to disease. *PLOS Pathogen* 13(7):e1006323. https://doi.org/10.1371/journal.ppat.1006323.

Morales, R., K. Abid, and C. Soto. 2007. The prion strain phenomenon: Molecular basis and unprecedented features. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1772(6):681-691. https://doi.org/10.1016/j.bbadis.2006.12.006.

Moreno, J.A., and G.C. Telling. 2018. Molecular Mechanisms of Chronic Wasting Disease Prion Propagation. *Cold Spring Harbor Perspectives in Medicine* 8(6). https://doi.org/10.1101/cshperspect.a024448.

Mori, J., N. Rivera, J. Novakofski, and N. Mateus-Pinilla. 2024. A review of chronic wasting disease (CWD) spread, surveillance, and control in the United States captive cervid industry. *Prion* 18(1):54-67. https://doi.org/10.1080/19336896.2024.2343220.

Mu, J.E., B.A. McCarl, A. Hagerman, and D. Bessler. 2015. Impacts of bovine spongiform encephalopathy and avian influenza on U.S. meat demand. *Journal of Integrative Agriculture* 14(6):1130-1141. https://doi.org/10.1016/S2095-3119(14)60996-5.

Muhammed, T.S., and S.K. Mathew. 2022. The disaster of misinformation: a review of research in social media. *International Journal of Data Science and Analytics* 13(4):271-285. https://doi.org/10.1007/s41060-022-00311-6.

Muhsin, S.A., A. Abdullah, E. Kobashigawa, M. Al-Amidie, S. Russell, M.Z. Zhang, S. Zhang, and M. Almasri. 2023. A microfluidic biosensor for the diagnosis of chronic wasting disease. *Microsystems & Nanoengineering* 9(1):104. https://doi.org/10.1038/s41378-023-00569-1.

Nagaoka, K., M. Yoshioka, N. Shimozaki, T. Yamamura, Y. Murayama, T. Yokoyama, and S. Mohri. 2010. Sensitive detection of scrapie prion protein in soil. *Biochemical and Biophysical Research Communications* 397(3):626-630. https://doi.org/10.1016/j.bbrc.2010.06.013.

Nalls, A.V., E. McNulty, C.E. Hoover, L.A. Pulscher, E.A. Hoover, and C.K. Mathiason. 2017. Infectious Prions in the Pregnancy Microenvironment of Chronic Wasting Disease-Infected Reeves' Muntjac Deer. *Journal of Virology* 91(15). https://doi.org/10.1128/jvi.00501-17.

Nalls, A.V., E. McNulty, J. Powers, D.M. Seelig, C. Hoover, N.J. Haley, J. Hayes-Klug, K. Anderson, P. Stewart, W. Goldmann, E.A. Hoover, and C.K. Mathiason. 2013. Mother to offspring transmission of chronic wasting disease in reeves' muntjac deer. *PLOS ONE* 8(8):e71844. https://doi.org/10.1371/journal.pone.0071844.

Nalls, A.V., E.E. McNulty, A. Mayfield, J.M. Crum, M.K. Keel, E.A. Hoover, M.G. Ruder, and C.K. Mathiason. 2021. Detection of Chronic Wasting Disease Prions in Fetal Tissues of Free-Ranging White-Tailed Deer. *Viruses* 13(12). https://doi.org/10.3390/v13122430.

National CWD Plan Implementation Committee. 2002. *Implementation Document for Plan for Assisting States, Federal Agencies, and Tribes in Managing Chronic Wasting Disease in Wild and Captive Cervids October 13, 2002.* https://govdocs.nebraska.gov/epubs/G1800/B023-2002.pdf.

National Resource Council (NRC). Committee on Key Challenge Areas for, Convergence Health, Board on Life, Sciences Division on, Earth, and Studies National Research Life, Council. 2014. The National Academies Collection: Reports funded by National Institutes of Health. In Convergence: Facilitating Transdisciplinary Integration of Life Sciences, Physical Sciences, Engineering, and Beyond. Washington (DC): National Academies Press.

National Resource Council (NRC). 2004. *Adaptive Management for Water Resources Project Planning.* Washington, DC: The National Academies Press. https://doi.org/10.17226/10972.

Needham, M., and J. Vaske. 2006. Beliefs about Chronic Wasting Disease Risks across Multiple States, Years, and Interest Groups. *Human Dimensions of Wildlife* 11:215-220. https://doi.org/10.1080/10871200600669932.

Needham, M.D., J.J. Vaske, and M.J. Manfredo. 2004. Hunters' Behavior and Acceptance of Management Actions Related to Chronic Wasting disease in Eight States. *Human Dimensions of Wildlife* 9:211-231. https://doi.org/10.1080/10871200490479990.

Ness, A., D. Zeng, A. Kuznetsova, A. Otero, C. Kim, K. Saboraki, S. Lingle, M. Pybus, J. Aiken, S. Gilch, and D. McKenzie. 2022. Chronic wasting disease prions in mule deer interdigital glands. *PLOS ONE* 17(10):e0275375. https://doi.org/10.1371/journal.pone.0275375.

New York State Department of Environmental Conservation (DEC), New York State Department of Agriculture and Markets (DAM), and Cornell University College of Veterinary Medicine Wildlife Health. 2018. New York State Interagency CWD Risk Minimization Plan. https://extapps.dec.ny.gov/docs/wildlife_pdf/cwdpreventionplan2018.pdf (accessed August 27, 2024).

Newsome, T.M., L. Boitani, G. Chapron, P. Ciucci, C.R. Dickman, J.A. Dellinger, J.V. López-Bao, R.O. Peterson, C.R. Shores, A.J. Wirsing, and W.J. Ripple. 2016. Food habits of the world's grey wolves. *Mammal Review* 46(4):255-269. https://doi.org/10.1111/mam.12067.

Nichols, T.A., B. Pulford, A.C. Wyckoff, C. Meyerett, B. Michel, K. Gertig, E.A. Hoover, J.E. Jewell, G.C. Telling, and M.D. Zabel. 2009. Detection of protease-resistant cervid prion protein in water from a CWD-endemic area. *Prion* 3(3):171-183. https://doi.org/10.4161/pri.3.3.9819.

Nichols, T.A., T.R. Spraker, T. Gidlewski, J.G. Powers, G.C. Telling, K.C. VerCauteren, and M.D. Zabel. 2012. Detection of prion protein in the cerebrospinal fluid of elk (Cervus canadensis nelsoni) with chronic wasting disease using protein misfolding cyclic amplification. *Journal of Veterinary Diagnostic Investigation* 24(4):746-9. https://doi.org/10.1177/1040638712448060.

Nichols, T.A., J.W. Fischer, T.R. Spraker, Q. Kong, and K.C. VerCauteren. 2015. CWD prions remain infectious after passage through the digestive system of coyotes (Canis latrans). *Prion* 9(5):367-75. https://doi.org/10.1080/19336896.2015.1086061.

Nobert, B.R., E.H. Merrill, M.J. Pybus, T.K. Bollinger, and Y.T. Hwang. 2016. Landscape connectivity predicts chronic wasting disease risk in Canada. *Journal of Applied Ecology* 53(5):1450-1459.

Nodelijk, G., H.J. van Roermund, L.J. van Keulen, B. Engel, P. Vellema, and T.J. Hagenaars. 2011. Breeding with resistant rams leads to rapid control of classical scrapie in affected sheep flocks. *Veterinary Research* 42(1):5. https://doi.org/10.1186/1297-9716-42-5.

Nonno, R., M.A. Di Bari, L. Pirisinu, C. D'Agostino, I. Vanni, B. Chiappini, S. Marcon, G. Riccardi, L. Tran, T. Vikøren, J. Våge, K. Madslien, G. Mitchell, G.C. Telling, S.L. Benestad, and U. Agrimi. 2020. Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America. *Proceedings of the National Academy of Sciences* 117(49):31417-31426. https://doi.org/10.1073/pnas.2013237117.

Nusser, S.M., W.R. Clark, D.L. Otis, and L. Huang. 2008. Sampling considerations for disease surveillance in wildlife populations. *Journal of Wildlife Management* 72(1):52-60. https://doi.org/10.2193/2007-317.

O'Hara Ruiz, M., A.C. Kelly, W.M. Brown, J.E. Novakofski, and N.E. Mateus-Pinilla. 2013. Influence of landscape factors and management decisions on spatial and temporal patterns of the transmission of chronic wasting disease transmission in white-tailed deer. *Geospatial Health* 8(1):215-27. https://doi.org/10.4081/gh.2013.68.

Olszowy, K.M., J. Lavelle, K. Rachfal, S. Hempstead, K. Drouin, J.M. Darcy 2nd, C. Reiber, and R.M. Garruto. 2014. Six-year follow-up of a point-source exposure to CWD contaminated venison in an Upstate New York community: risk behaviours and health outcomes 2005-2011. *Public Health* 128(9):860-8. https://doi.org/10.1016/j.puhe.2014.06.012.

Organ, J., V. Geist, S. Mahoney, S. Williams, G. Batcheller, T. Decker, R. Carmichael, P. Nanjappa, R. Regan, R. Medellín, R. Cantu, R. McCabe, S. Craven, and G. Vecellio. The North American Model of Wildlife Conservation. 2012.

Organ, J.F., T.A. Decker, and T.M. Lama. 2016. The North American model and captive cervid facilities-what is the threat? Wildlife Society Bulletin. 40(1):10-13.

Orge, L., A. Galo, C. Machado, C. Lima, C. Ochoa, J. Silva, M. Ramos, and J.P. Simas. 2004. Identification of putative atypical scrapie in sheep in Portugal. *Journal of General Virology* 85(Pt 11):3487-3491. https://doi.org/10.1099/vir.0.80246-0.

O'Rourke, K.I., T.E. Besser, M.W. Miller, T.F. Cline, T.R. Spraker, A.L. Jenny, M.A. Wild, G.L. Zebarth, and E.S. Williams. 1999. PrP genotypes of captive and free-ranging Rocky Mountain elk (Cervus elaphus nelsoni) with chronic wasting disease. *Journal of General Virology* 80(Pt 10):2765-2679. https://doi.org/10.1099/0022-1317-80-10-2765.

O'Rourke, K.I., T.R. Spraker, L.K. Hamburg, T.E. Besser, K.A. Brayton, and D.P. Knowles. 2004. Polymorphisms in the prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer. *Journal of General Virology* 85(Pt 5):1339-1346. https://doi.org/10.1099/vir.0.79785-0.

O'Rourke, K.I., T.R. Spraker, D. Zhuang, J.J. Greenlee, T.E. Gidlewski, and A.N. Hamir. 2007. Elk with a long incubation prion disease phenotype have a unique PrPd profile. *Neuroreport* 18(18):1935-8. https://doi.org/10.1097/WNR.0b013e3282f1ca2f.

Orrù, C.D., B.R. Groveman, L.D. Raymond, A.G. Hughson, R. Nonno, W. Zou, B. Ghetti, P. Gambetti, and B. Caughey. 2015. Bank Vole Prion Protein as an Apparently Universal Substrate for RT-QuIC-Based Detection and Discrimination of Prion Strains. *PLOS Pathogens* 11(6):e1004983. https://doi.org/10.1371/journal.ppat.1004983.

Orrù, C.D., B.R. Groveman, A.G. Hughson, M. Manca, L.D. Raymond, G.J. Raymond, K.J. Campbell, K.J. Anson, A. Kraus, and B. Caughey. 2017. RT-QuIC Assays for Prion Disease Detection and Diagnostics. *Methods in Molecular Biology* 1658:185-203. https://doi.org/10.1007/978-1-4939-7244-9_14.

Osnas, E.E., D.M. Heisey, R.E. Rolley, and M.D. Samuel. 2009. Spatial and temporal patterns of chronic wasting disease: fine-scale mapping of a wildlife epidemic in Wisconsin. *Journal of Applied Ecology* 19(5):1311-22. https://doi.org/10.1890/08-0578.1.

Osterholm, M.T., C.J. Anderson, M.D. Zabel, J.M. Scheftel, K.A. Moore, and B.S. Appleby. 2019. Chronic Wasting Disease in Cervids: Implications for Prion Transmission to Humans and Other Animal Species. *mBio* 10(4). https://doi.org/doi:10.1128/mbio.01091-19.

Otero, A., C. Duque Velásquez, C. Johnson, A. Herbst, R. Bolea, J.J. Badiola, J. Aiken, and D. McKenzie. 2019. Prion protein polymorphisms associated with reduced CWD susceptibility limit peripheral

PrP(CWD) deposition in orally infected white-tailed deer. *BMC Veterinary Research* 15(1):50. https://doi.org/10.1186/s12917-019-1794-z.

Otero, A., C. Duque Velásquez, J. Aiken, and D. McKenzie. 2021. Chronic wasting disease: a cervid prion infection looming to spillover. *Veterinary Research* 52(1):115. https://doi.org/10.1186/s13567-021-00986-y.

Otero, A., C. Duque Velasquez, D. McKenzie, and J. Aiken. 2023. Emergence of CWD strains. *Cell & Tissue Research* 392(1):135-148. https://doi.org/10.1007/s00441-022-03688-9.

Outlaw J.L., D.P. Anderson, M.L. Earle, and J.W. Richardson. 2017. Economic impact of the Texas deer breeding and hunting operations. Agricultural & Food Policy Center, Department of Agricultural Economics, Texas A&M AgriLife Research, Texas A&M AgriLife Extension Service, Texas A&M University, Research Report 17-3. College Station, Texas..

Palaiokostas, C., S. Cariou, A. Bestin, J.-S. Bruant, P. Haffray, T. Morin, J. Cabon, F. Allal, M. Vandeputte, and R.D. Houston. 2018. Genome-wide association and genomic prediction of resistance to viral nervous necrosis in European sea bass (Dicentrarchus labrax) using RAD sequencing. *Genetics Selection Evolution* 50(1):30. https://doi.org/10.1186/s12711-018-0401-2.

Parchi, P., R. Castellani, S. Capellari, B. Ghetti, K. Young, S.G. Chen, M. Farlow, D.W. Dickson, A.A.F. Sima, J.Q. Trojanowski, R.B. Petersen, and P. Gambetti. 1996. Molecular basis of phenotypic variability in sporadc creudeldt-jakob disease. *Annals of Neurology* 39(6):767-778. https://doi.org/https://doi.org/10.1002/ana.410390613.

Parchi, P., S. Capellari, S.G. Chen, R.B. Petersen, P. Gambetti, N. Kopp, P. Brown, T. Kitamoto, J. Tateishi, A. Giese, and H. Kretzschmar. 1997. Typing prion isoforms. *Nature* 386(6622):232-233. https://doi.org/10.1038/386232a0.

Parchi, P., W. Zou, W. Wang, P. Brown, S. Capellari, B. Ghetti, N. Kopp, W.J. Schulz-Schaeffer, H.A. Kretzschmar, M.W. Head, J.W. Ironside, P. Gambetti, and S.G. Chen. 2000. Genetic influence on the structural variations of the abnormal prion protein. *Proceedings of the National Academy of Sciences* 97(18):10168-10172. https://doi.org/doi:10.1073/pnas.97.18.10168.

Park, J.H., Y.G. Choi, S.J. Park, H.S. Choi, E.K. Choi, and Y.S. Kim. 2018. Ultra-efficient Amplification of Abnormal Prion Protein by Modified Protein Misfolding Cyclic Amplification with Electric Current. *Molecular Neurobiology* 55(2):1630-1638. https://doi.org/10.1007/s12035-017-0431-8.

Parlee, B., K. Ahkimnachie, H. Cunningham, M. Jordan, and E. Goddard. 2021. "It's important to know about this" - risk communication and the impacts of chronic wasting disease on indigenous food systems in Western Canada. *Environmental Science & Policy* 123:190-201. https://doi.org/10.1016/j.envsci.2021.05.012.

Pasick, J. 2004. Application of DIVA vaccines and their companion diagnostic tests to foreign animal disease eradication. *Animal Health Research Reviews* 5(2):257-62. https://doi.org/10.1079/ahr200479.

Pattison, I.H., and G.C. Millson. 1961. Scrapie produced experimentally in goats with special reference to the clinical syndrome. *Journal of Comparative Pathology* 71:101-9. https://doi.org/10.1016/s0368-1742(61)80013-1.

Pattison-Williams, J.K., L. Xie, W.L. Adamowicz, M. Pybus, and A. Hubbs. 2020. An empirical analysis of hunter response to chronic wasting disease in Alberta. *Human Dimensions of Wildlife* 25(6):575-589. https://doi.org/10.1080/10871209.2020.1780351.

Perloff, J.M. Microeconomics 5th ed. 2009. Pearson Education International Edition.

Perrott, M.R., C.J. Sigurdson, G.L. Mason, and E.A. Hoover. 2012. Evidence for distinct chronic wasting disease (CWD) strains in experimental CWD in ferrets. *Journal of General Virology* 93(Pt 1):212-221. https://doi.org/10.1099/vir.0.035006-0.

Perucchini, M., K. Griffin, M.W. Miller, and W. Goldmann. 2008. PrP genotypes of free-ranging wapiti (Cervus elaphus nelsoni) with chronic wasting disease. *Journal of General Virology* 89(Pt 5):1324-1328. https://doi.org/10.1099/vir.0.83424-0.

Pesch, V., J.M. Flores-Fernandez, S. Reithofer, L. Ma, P. Özdüzenciler, Y. Busch, A. Sriraman, Y. Wang, S. Amidian, C.V.M. Kroepel, L. Müller, Y. Lien, O. Rudtke, B. Frieg, G.F. Schröder, H. Wille, and G. Tamgüney. 2024. Vaccination with structurally adapted fungal protein fibrils induces immunity to Parkinson's disease. *Brain* 147(5):1644-1652. https://doi.org/10.1093/brain/awae061.

Pesendorfer, W. 2006. Behavioral Economics Comes of Age: A Review Essay on "Advances in Behavioral Economics". *Journal of Economic Literature* 44(3):712-721.

Petchenik, J. 2006. Landowner, Responses to Harvest Incentives in Wisconsin's Southwest Chronic Wasting Disease Eradication Zone. *Human Dimensions in Wildlife* 11(3):225-226. https://doi.org/10.1080/10871200600669957.

Petersen, E., N. Petrosillo, and M. Koopmans. 2018. Emerging infections-an increasingly important topic: review by the Emerging Infections Task Force. *Clinical Microbiology and Infection* 24(4):369-375. https://doi.org/10.1016/j.cmi.2017.10.035.

Petigara, M., C. Dridi, and J. Unterschultz. 2011. The economic impacts of chronic wasting disease and bovine spongiform encephalopathy in Alberta and the rest of Canada. *Journal of Toxicology and Environmental Health* 74 Part A(22-24):1609-20. https://doi.org/10.1080/15287394.2011.618987.

Picasso-Risso, C., M.D. Schwabenlander, G. Rowden, M. Carstensen, J.C. Bartz, P.A. Larsen, and T.M. Wolf. 2022. Assessment of Real-Time Quaking-Induced Conversion (RT-QuIC) Assay, Immunohistochemistry and ELISA for Detection of Chronic Wasting Disease under Field Conditions in White-Tailed Deer: A Bayesian Approach. *Pathogens* 11(5). https://doi.org/10.3390/pathogens11050489.

Pilon, J.L., J.C. Rhyan, L.L. Wolfe, T.R. Davis, M.P. McCollum, K.I. O'Rourke, T.R. Spraker, K.C. VerCauteren, M.W. Miller, T. Gidlewski, T.A. Nichols, L.A. Miller, and P. Nol. 2013. Immunization with a synthetic peptide vaccine fails to protect mule deer (Odocoileus hemionus) from chronic wasting disease. *Journal of Wildlife Diseases* 49:694-698.

Pirisinu, L., L. Tran, B. Chiappini, I. Vanni, M.A. Di Bari, G. Vaccari, T. Vikøren, K.I. Madslien, J. Våge, T. Spraker, G. Mitchell, A. Balachandran, T. Baron, C. Casalone, C.M. Rolandsen, K.H. Røed, U. Agrimi, R. Nonno, and S.L. Benestad. 2018. Novel Type of Chronic Wasting Disease Detected in Moose (Alces alces), Norway. *Emerging Infectious Diseases* 24(12):2210-2218. https://doi.org/10.3201/eid2412.180702.

Plummer, I.H., S.D. Wright, C.J. Johnson, J.A. Pedersen, and M.D. Samuel. 2017. Temporal patterns of chronic wasting disease prion excretion in three cervid species. *Journal of General Virology* 98(7):1932-1942. https://doi.org/10.1099/jgv.0.000845.

Plummer, I.H., C.J. Johnson, A.R. Chesney, J.A. Pedersen, and M.D. Samuel. 2018. Mineral licks as environmental reservoirs of chronic wasting disease prions. *PLOS ONE* 13(5):e0196745. https://doi.org/10.1371/journal.pone.0196745.

Potapov, A., E. Merrill, M. Pybus, and M.A. Lewis. 2016. Chronic Wasting Disease: Transmission Mechanisms and the Possibility of Harvest Management. *PLOS ONE* 11(3):e0151039. https://doi.org/10.1371/journal.pone.0151039.

Priadka, P., B. Moses, C. Kozmik, S. Kell, and J.N. Popp. 2022. Impacts of harvested species declines on Indigenous Peoples' food sovereignty, well-being and ways of life: a case study of Anishinaabe perspectives and moose. *Ecology & Society* 27(1):1-15. https://doi.org/10.5751/ES-12995-270130.

Prion 2016 Animal Prion Disease Workshop Abstracts. 2016. *Prion* 10 Supplement 1:S15-21. https://doi.org/10.1080/19336896.2016.1163048.

Pritzkow, S., R. Morales, F. Moda, U. Khan, G.C. Telling, E. Hoover, and C. Soto. 2015. Grass plants bind, retain, uptake, and transport infectious prions. *Cell Reports* 11(8):1168-75. https://doi.org/10.1016/j.celrep.2015.04.036.

Pritzkow, S., R. Morales, A. Lyon, L. Concha-Marambio, A. Urayama, and C. Soto. 2018. Efficient prion disease transmission through common environmental materials. *Journal of Biological Chemistry* 293 (9):3363-3373. https://doi.org/10.1074/jbc.M117.810747.

Pritzkow, S., R. Morales, M. Camacho, and C. Soto. 2021. Uptake, Retention, and Excretion of Infectious Prions by Experimentally Exposed Earthworms. *Emerging Infectious Diseases* 27(12):3151-3154. https://doi.org/10.3201/eid2712.204236.

Prusiner, S.B. 1982. Novel Proteinaceous Infectious Particles Cause Scrapie. *Science* 216(4542):136-144. https://doi.org/doi:10.1126/science.6801762.

Prusiner, S.B., M. Scott, D. Foster, K.M. Pan, D. Groth, C. Mirenda, M. Torchia, S.L. Yang, D. Serban, G.A. Carlson, and et al. 1990. Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* 63(4):673-86. https://doi.org/10.1016/0092-8674(90)90134-z.

Prusiner, S.B. 1991. Molecular biology of prion diseases. *Science* 252(5012):1515-22. https://doi.org/10.1126/science.1675487.

Prusiner, S.B. 1998. Prions. *Proceedings of the National Academy of Sciences* 95(23):13363-83. https://doi.org/10.1073/pnas.95.23.13363.

Race, R., M. Oldstone, and B. Chesebro. 2000. Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: role of prion protein expression in peripheral nerves and spleen. *Journal of Virology* 74(2):828-33. https://doi.org/10.1128/jvi.74.2.828-833.2000.

Race, B.L., K.D. Meade-White, A. Ward, J. Jewell, M.W. Miller, E.S. Williams, B. Chesebro, and R.E. Race. 2007. Levels of abnormal prion protein in deer and elk with chronic wasting disease. *Emerging Infectious Diseases* 13(6):824-30. https://doi.org/10.3201/eid1306.070186.

Race, B., K. Meade-White, R. Race, and B. Chesebro. 2009a. Prion infectivity in fat of deer with chronic wasting disease. *Journal of Virology* 83(18):9608-10. https://doi.org/10.1128/jvi.01127-09.

Race, B., K.D. Meade-White, M.W. Miller, K.D. Barbian, R. Rubenstein, G. LaFauci, L. Cervenakova, C. Favara, D. Gardner, D. Long, M. Parnell, J. Striebel, S.A. Priola, A. Ward, E.S. Williams, R. Race, and B. Chesebro. 2009b. Susceptibilities of nonhuman primates to chronic wasting disease. *Emerging Infectious Diseases* 15(9):1366-76. https://doi.org/10.3201/eid1509.090253.

Race, B., C. Baune, K. Williams, J.F. Striebel, A.G. Hughson, and B. Chesebro. 2022. Second passage experiments of chronic wasting disease in transgenic mice overexpressing human prion protein. *Veterinary Research* 53(1):111. https://doi.org/10.1186/s13567-022-01130-0.

Race, B., K. Williams, and B. Chesebro. 2019. Transmission studies of chronic wasting disease to transgenic mice overexpressing human prion protein using the RT-QuIC assay. *Veterinary Research* 50(1):6. https://doi.org/10.1186/s13567-019-0626-2.

Race, B., K. Williams, C.D. Orrú, A.G. Hughson, L. Lubke, and B. Chesebro. 2018. Lack of Transmission of Chronic Wasting Disease to Cynomolgus Macaques. *Journal of Virology* 92(14). https://doi.org/10.1128/jvi.00550-18.

Raudabaugh, D.B., Y. Ishida, N.J. Haley, W.M. Brown, J. Novakofski, A.L. Roca, and N.E. Mateus-Pinilla. 2022. County-wide assessments of Illinois white-tailed deer (Odocoileus virginianus) prion protein gene variation using improved primers and potential implications for management. *PLOS ONE* 17(11):e0274640. https://doi.org/10.1371/journal.pone.0274640.

Raymond, G.J., L.D. Raymond, K.D. Meade-White, A.G. Hughson, C. Favara, D. Gardner, E.S. Williams, M.W. Miller, R.E. Race, and B. Caughey. 2007. Transmission and adaptation of chronic wasting disease to hamsters and transgenic mice: evidence for strains. *Journal of Virology* 81(8):4305-14. https://doi.org/10.1128/jvi.02474-06.

Richt, J.A., P. Kasinathan, A.N. Hamir, J. Castilla, T. Sathiyaseelan, F. Vargas, J. Sathiyaseelan, H. Wu, H. Matsushita, J. Koster, S. Kato, I. Ishida, C. Soto, J.M. Robl, and Y. Kuroiwa. 2007. Production of cattle lacking prion protein. *Nature Biotechnology* 25(1):132-8. https://doi.org/10.1038/nbt1271.

Robinson, S.J., M.D. Samuel, K.I. O'Rourke, and C.J. Johnson. 2012. The role of genetics in chronic wasting disease of North American cervids. *Prion* 6(2):153-62. https://doi.org/10.4161/pri.19640.

Rowden, G.R., C. icasso-Risso, M. Li, M.D. Schwabenlander, T.M. Wolf, and P.A. Larsen. 2023. Standardization of Data Analysis for RT-QuIC-Based Detection of Chronic Wasting Disease. *Pathogens* 12(2). https://doi.org/10.3390/pathogens12020309.

Rubino, E.C., and C. Serenari. 2022. Landowner perceptions of and preferences for chronic wasting disease management. *Environmental Challenges*. 8: 100582. https://doi.org/10.1016/j.envc.2022.100582.

Ruder, Mark G., John R. Fischer, and Michael W. Miller. 2024. Reinterpreting Chronic Wasting Disease Emergence in the USA in Light of Historical Surveillance Limitations. *Journal of Wildlife Diseases*. https://doi.org/10.7589/JWD-D-24-00077.

Russell, R.E., J.A. Gude, N.J. Anderson, and J.M. Ramsey. 2015. Identifying priority chronic wasting disease surveillance areas for mule deer in Montana. *The Journal of Wildlife Management* 79(6):989-997. https://doi.org/10.1002/jwmg.914.

Rutala, W.A., and D.J. Weber. 2010. Guideline for Disinfection and Sterilization of Prion-Contaminated Medical Instruments. *Infection Control & Hospital Epidemiology* 31(2):107-117. https://doi.org/10.1086/650197.

Rutjes, A.W., J.B. Reitsma, A. Coomarasamy, K.S. Khan, and P.M. Bossuyt. 2007. Evaluation of diagnostic tests when there is no gold standard. A review of methods. *Health Technology Assessment Reports* 11(50):iii, ix-51. https://doi.org/10.3310/hta11500.

Saá, P., J. Castilla, and C. Soto. 2005. Cyclic amplification of protein misfolding and aggregation. *Methods in Molecular Biology* 299:53-65. https://doi.org/10.1385/1-59259-874-9:053.

Saá, P., J. Castilla, and C. Soto. 2006. Ultra-efficient replication of infectious prions by automated protein misfolding cyclic amplification. *Journal of Biological Chemistry* 281(46):35245-52. https://doi.org/10.1074/jbc.M603964200.

Saah, A.J., and D.R. Hoover. 1997. "Sensitivity" and "specificity" reconsidered: the meaning of these terms in analytical and diagnostic settings. *Annals of Internal Medicine* 126(1):91-4. https://doi.org/10.7326/0003-4819-126-1-199701010-00026.

Safar, J., P.P. Roller, D.C. Gajdusek, and C.J. Gibbs. 1993. Conformational transitions, dissociation, and unfolding of scrapie amyloid (prion) protein. *Journal of Biological Chemistry* 268(27):20276-20284. https://doi.org/10.1016/S0021-9258(20)80725-X.

Safar, J.G., M.D. Geschwind, C. Deering, S. Didorenko, M. Sattavat, H. Sanchez, A. Serban, M. Vey, H. Baron, K. Giles, B.L. Miller, S.J. Dearmond, and S.B. Prusiner. 2005. Diagnosis of human prion disease. *Proceedings of the National Academy of Sciences* 102(9):3501-6. https://doi.org/10.1073/pnas.0409651102.

Safar, J.G., X. Xiao, M.E. Kabir, S. Chen, C. Kim, T. Haldiman, Y. Cohen, W. Chen, M.L. Cohen, and W.K. Surewicz. 2015. Structural Determinants of Phenotypic Diversity and Replication Rate of Human Prions. *PLOS Pathogens* 11(4):e1004832. https://doi.org/10.1371/journal.ppat.1004832.

Sailer, A., H. Büeler, M. Fischer, A. Aguzzi, and C. Weissmann. 1994. No propagation of prions in mice devoid of PrP. *Cell* 77(7):967-8. https://doi.org/10.1016/0092-8674(94)90436-7.

Samuel, M.D., D.O. Joly, M.A. Wild, S.D. Wright, D.L. Otis, R.W. Werge, and M.W. Miller. 2003. Surveillance strategies for detecting Chronic Wasting Disease in free-ranging deer and elk: Results of a CWD surveillance workshop. U.S. Geological Survey, National Wildlife Health Center. https://pubs.usgs.gov/confpub/70006758/cwd_surveillance_strategies.pdf.

Sandberg, M.K., H. Al-Doujaily, C.J. Sigurdson, M. Glatzel, C. Malley, C. Powell, E.A. Asante, J.M. Linehan, S. Brandner, J.D.F. Wadsworth, and J. Collinge. 2010. Chronic wasting disease prions are not transmissible to transgenic mice overexpressing human prion protein. *Journal of General Virology* 91(10):2651-2657. https://doi.org/10.1099/vir.0.024380-0.

Sargeant, G.A., D.C. Weber, and D.E. Roddy. 2011. Implications of chronic wasting disease, cougar predation, and reduced recruitment for elk management. *The Journal of Wildlife Management* 75(1):171-177. https://doi.org/10.1002/jwmg.27.

Saskatchewan Ministry of Environment. 2020. *Saskatchewan Wildlife Management Report 2020.* Fish and Wildlife Technical Report 2020.

Saunders, S.E., S.L. Bartelt-Hunt, and J.C. Bartz. 2008. Prions in the environment: occurrence, fate and mitigation. *Prion*. 2 (4): 162-9. https://doi.org/10.4161/pri.2.4.7951.

Saunders, S.E., J.C. Bartz, and S.L. Bartelt-Hunt. 2009. Prion Protein Adsorption to Soil in a Competitive Matrix Is Slow and Reduced. *Environmental Science & Technology* 43(20):7728-7733. https://doi.org/10.1021/es901385t.

Saunders, S.E., J.C. Bartz, and S.L. Bartelt-Hunt. 2012. Soil-mediated prion transmission: is local soil-type a key determinant of prion disease incidence? *Chemosphere* 87(7):661-7. https://doi.org/10.1016/j.chemosphere.2011.12.076.

Schauber, E.M., D.J. Storm, and C.K. Nielsen. 2007. Effects of Joint Space Use and Group Membership on Contact Rates Among White-Tailed Deer. *The Journal of Wildlife Management* 71(1):155-163. https://doi.org/https://doi.org/10.2193/2005-546.

Schauber, E.M., C.K. Nielsen, L.J. Kjær, C.W. Anderson, and D.J. Storm. 2015. Social affiliation and contact patterns among white-tailed deer in disparate landscapes: implications for disease transmission. *Journal of Mammalogy* 96(1):16-28. https://doi.org/10.1093/jmammal/gyu027.

Schmidt, A., A.M. Groh, J.S. Frick, M.J.G.T. Vehreschild, and K.U. Ludwig. 2022. Genetic Predisposition and the Variable Course of Infections Diseases. *Deutsches Ärzteblatt International* 119(8):117-123. https://doi.org/10.3238/arztebl.m2022.0105.

Schrage, M. Personal Communication, November 29th, 2023.
Schroeder, S.A., A. Landon, L.J. Cornicelli, D.C. Fulton, and L. McInenly. 2021. Institutional trust, beliefs, and evaluation of regulations, and management of chronic wasting disease (CWD). *Human Dimensions of Wildlife* 26(3):228-244. https://doi.org/10.1080/10871209.2020.1808915.
Schultze, M.L., A. Horn-Delzer, L. Glaser, A. Hamberg, D. Zellner, T.M. Wolf, and S.J. Wells. 2023. Herd-level risk factors associated with chronic wasting disease-positive herd status in Minnesota, Pennsylvania, and Wisconsin cervid herds. *Preventive Veterinary Medicine* 218:106000. https://doi.org/10.1016/j.prevetmed.2023.106000.
Schuler, K.L., Hollinghead, N., Abbott, R.C., Miller, L., Hurst, J., and K. Hynes. 2022. Risk-Weighted Surveillance for Chronic Wasting Disease in New York. Cornell Wildlife Health Lab and New York State Department of Environmental Conservation. https://extapps.dec.ny.gov/docs/wildlife_pdf/cwdsurplan.pdf (accessed August 27, 2024).
Schwabenlander, M.D., N. Potts, S. Moore, P.A. Larsen, L.A. Bernstein, and T.M. Wolf. 2022. Upper Midwest tribal natural resource managers' perspectives on chronic wasting disease outreach, surveillance, and management. *Conservation Science and Practice* 4(7):e12710. https://doi.org/10.1111/csp2.12710.
Schwabenlander, M., C. Picasso-Risso, S. Gresch, M. Milstein, G. Rowden, E. Hildebrand, P. Hagen, M. Lockwood, J. Hediger, M. Cherry, D. Hewitt, Q. Yuan, J. Bartz, T. Wolf, and P. Larsen. 2023a. CWD Sentinels: Detecting Environmental Prion Protein (ePrP) Via Surfaces for the Early Discovery of Chronic Wasting Disease. Southeast Deer Study Group Annual Meeting.
Schwabenlander, M., C. Picasso-Risso, S. Gresch, M. Milstein, G. Rowden, E. Hildebrand, P. Hagen, M. Lockwood, J. Hediger, M. Cherry, D. Hewitt, Q. Yuan, J. Bartz, T. Wolf, and P. Larsen. 2023b. Application of Methods for Detecting Environmental Prion Protein (ePrP) Via Surfaces to Managing Chronic Wasting Disease [Conference Presentation]. 4th International Chronic Wasting Disease Symposium.
Schwabenlander, M.D., J.C. Bartz, M. Carstensen, A. Fameli, L. Glaser, R.J. Larsen, M. Li, R.L. Shoemaker, G. Rowden, S. Stone, W.D. Walter, T.M. Wolf, and P.A. Larsen. 2024. Prion forensics: a multidisciplinary approach to investigate CWD at an illegal deer carcass disposal site. *Prion* 18(1):72-86. https://doi.org/10.1080/19336896.2024.2343298.
Schwartz, M.A. 2024. The 574 Federally Recognized Indian Tribes in the United States. R47414. Congressional Research Service.
Seabury, C.M., D.L. Oldeschulte, E.K. Bhattarai, D. Legare, P.J. Ferro, R.P. Metz, C.D. Johnson, M.A. Lockwood, and T.A. Nichols. 2020. Accurate Genomic Predictions for Chronic Wasting Disease in U.S. White-Tailed Deer. *G3 (Bethesda)*. 10 (4): 1433-1441. https://doi.org/10.1534/g3.119.401002.
Seabury, C.M., M.A. Lockwood, and T.A. Nichols. 2022. Genotype by environment interactions for chronic wasting disease in farmed US white-tailed deer. *G3 Genes|Genomes|Genetics* 12(7). https://doi.org/10.1093/g3journal/jkac109.
Secker, T.J., R. Hervé, and C.W. Keevil. 2011. Adsorption of prion and tissue proteins to surgical stainless-steel surfaces and the efficacy of decontamination following dry and wet storage conditions. *Journal of Hospital Infection* 78(4):251-255. https://doi.org/10.1016/j.jhin.2011.03.021.
Seidl, A.F., and S.R. Koontz. 2004. Potential Economic Impacts of Chronic Wasting Disease in Colorado. *Human Dimensions of Wildlife.* 9(3): 241-245. https://doi.org/10.1080/10871200490480042.
Selariu, A., J.G. Powers, A. Nalls, M. Brandhuber, A. Mayfield, S. Fullaway, C.A. Wyckoff, W. Goldmann, M.M. Zabel, M.A. Wild, E.A. Hoover, and C.K. Mathiason. 2015. In utero transmission and tissue distribution of chronic wasting disease-associated prions in free-ranging Rocky Mountain elk. *Journal of General Virology* 96(11):3444-3455. https://doi.org/10.1099/jgv.0.000281.
Shikiya, R.A., A.E. Kincaid, J.C. Bartz, and T.J. Bourret. 2020. Failure To Detect Prion Infectivity in Ticks following Prion-Infected Blood Meal. *mSphere* 5(5). https://doi.org/10.1128/mSphere.00741-20.
Siemer, W.F., T.B. Lauber, and R.C. Stedman. 2020. New York hunters' perceptions of chronic wasting disease. Department of Natural Resources, Cornell University, College of Agriculture and Life Science. Ithaca, NY.
Sigurdson, C.J., E.S. Williams, M.W. Miller, T.R. Spraker, K.I. O'Rourke, and E.A. Hoover. 1999. Oral transmission and early lymphoid tropism of chronic wasting disease PrPres in mule deer fawns

(Odocoileus hemionus). *Journal of General Virology* 80(Pt 10):2757-2764. https://doi.org/10.1099/0022-1317-80-10-2757.

Sigurdson, C.J., T.R. Spraker, M.W. Miller, B. Oesch, and E.A. Hoover. 2001. PrP(CWD) in the myenteric plexus, vagosympathetic trunk and endocrine glands of deer with chronic wasting disease. *Journal of General Virology* 82(Pt 10):2327-2334. https://doi.org/10.1099/0022-1317-82-10-2327.

Sigurdson, C.J., C. Barillas-Mury, M.W. Miller, B. Oesch, L.J.M. van Keulen, J.P.M. Langeveld, and E.A. Hoover. 2002. PrP(CWD) lymphoid cell targets in early and advanced chronic wasting disease of mule deer. *Journal of General Virology* 83(Pt 10):2617-2628. https://doi.org/10.1099/0022-1317-83-10-2617.

Sigurdson, C.J., C.K. Mathiason, M.R. Perrott, G.A. Eliason, T.R. Spraker, M. Glatzel, G. Manco, J.C. Bartz, M.W. Miller, and E.A. Hoover. 2008. Experimental chronic wasting disease (CWD) in the ferret. *Journal of Comparative Pathology* 138(4):189-96. https://doi.org/10.1016/j.jcpa.2008.01.004.

Silberberg, E. 1972. Duality and the Many Consumer's Surpluses. *The American Economic Review* 62(5):942-952.

Silbernagel, E.R., N.K. Skelton, C.L. Waldner, and T.K. Bollinger. 2011. Interaction among deer in a chronic wasting disease endemic zone. *The Journal of Wildlife Management* 75(6):1453-1461. https://doi.org/10.1002/jwmg.172.

Sisó, S., M. Jeffrey, and L. González. 2010. Sensory circumventricular organs in health and disease. *Acta Neuropathology* 120(6):689-705. https://doi.org/10.1007/s00401-010-0743-5.

Sjöberg, L. 2000a. Factors in Risk Perception. *Risk Analysis* 20(1):1-12. https://doi.org/10.1111/0272-4332.00001.

Sjöberg, L. 2000b. Perceived Risk and Tampering with Nature. *Journal of Risk Research* 3(4):353-367. https://doi.org/10.1080/13669870050132568.

Slate, D., T.P. Algeo, K.M. Nelson, R.B. Chipman, D. Donovan, J.D. Blanton, M. Niezgoda, and C.E. Rupprecht. 2009. Oral rabies vaccination in North America: opportunities, complexities, and challenges. *PLOS Neglected Tropical Diseases* 3(12):e549. https://doi.org/10.1371/journal.pntd.0000549.

Smith, C.B., C.J. Booth, and J.A. Pedersen. 2011. Fate of prions in soil: a review. *Journal of Environmental Quality* 40(2):449-61. https://doi.org/10.2134/jeq2010.0412.

Smith, R., K. Chen, D. Winner, S. Friedhoff, and C. Wardle. 2023. A Systematic Review Of COVID-19 Misinformation Interventions: Lessons Learned. *Health Affairs*. 42 (12): 1738-1746. https://doi.org/10.1377/hlthaff.2023.00717.

Smolko, P., D. Seidel, M. Pybus, A. Hubbs, M. Ball, and E. Merrill. 2021. Spatio-temporal changes in chronic wasting disease risk in wild deer during 14 years of surveillance in Alberta, Canada. *Preventive Veterinary Medicine* 197:105512. https://doi.org/10.1016/j.prevetmed.2021.105512.

Sohn, H. J. 2018. Chronic wasting disease situation in the Republic of Korea. Presentation to the 9th International Deer Biology Conference, August 6, 2018. https://vimeo.com/user37284086/review/320249859/7eb92d8eee.

Sohn, H.J., J.H. Kim, K.S. Choi, J.J. Nah, Y.S. Joo, Y.H. Jean, S.W. Ahn, O.K. Kim, D.Y. Kim, and A. Balachandran. 2002. A case of chronic wasting disease in an elk imported to Korea from Canada. The *Journal of Veterinary Medical Science* 64(9):855-8. https://doi.org/10.1292/jvms.64.855.

Sohn, H.J., G. Mitchell, Y.H. Lee, H.J. Kim, K.J. Park, A. Staskevicus, I. Walther, A. Soutyrine, and A. Balachandran. 2020. Experimental oral transmission of chronic wasting disease to sika deer (Cervus nippon). *Prion* 14(1):271-277. https://doi.org/10.1080/19336896.2020.1857038.

Sola, D., L. Tran, J. Våge, K. Madslien, T.T. Vuong, S.L. Korpenfelt, E.O. Ågren, G. Averhed, M. Nöremark, K. Sören, M. Isaksson, C. Acín, J.J. Badiola, D. Gavier-Wildén, and S.L. Benestad. 2023. Heterogeneity of pathological prion protein accumulation in the brain of moose (*Alces alces*) from Norway, Sweden and Finland with chronic wasting disease. *Veterinary Research* 54(74). https://doi.org/10.1186/s13567-023-01208-3.

Song, H., K.A. McComas, and K.L. Schuler. 2019. Hunters' responses to urine-based scent bans tackling chronic wasting disease. *The Journal of Wildlife Management* 83(2):457-466. https://doi.org/10.1002/jwmg.21593.

Sorensen, A., F.M. van Beest, and R.K. Brook. 2014. Impacts of wildlife baiting and supplemental feeding on infectious disease transmission risk: A synthesis of knowledge. *Preventive Veterinary Medicine* 113(4):356-363. https://doi.org/10.1016/j.prevetmed.2013.11.010.

Soto, C., G.P. Saborio, and L. Anderes. 2002. Cyclic amplification of protein misfolding: application to prion-related disorders and beyond. *Trends in Neurosciences* 25(8):390-4. https://doi.org/10.1016/s0166-2236(02)02195-1.

Soto, P., J.H. Reed, M. Lockwood, and R. Morales. 2023a. Chronic wasting disease (CWD) detection in environmental and biological samples from a taxidermy site. 4th International Chronic Wasting Disease Symposium.

Soto, P., F. Bravo-Risi, C. Soto, and R. Morales. 2023b. Carrot plants as potential vectors for CWD transmission. 4th International CWD Symposium.

Soto, P., F. Bravo-Risi, R. Benavente, M.J. Bodenchuk, P. Whitley, C. Turnage, T.A. Nichols, T. Spraker, V.R Brown, and R. Morales. 2023c. Detection of infectious prions in tissues of feral hogs. 4th International CWD Symposium.

Soto, P., F. Bravo-Risi, R. Benavente, S. Lichtenberg, M. Lockwood, J.H. Reed, and R. Morales. 2023d. Identification of chronic wasting disease prions in decaying tongue tissues from exhumed white-tailed deer. *mSphere* 8(5):e00272-23. https://doi.org/doi:10.1128/msphere.00272-23. https://journals.asm.org/doi/abs/10.1128/msphere.00272-23.

Soto, P., F. Bravo-Risi, C. Kramm, N. Gamez, R. Benavente, D.L. Bonilla, J.H. Reed, M. Lockwood, T.R. Spraker, T. Nichols, and R. Morales. 2024. Nasal bots carry relevant titers of CWD prions in naturally infected white-tailed deer. *European Molecular Biology Organization Reports* 25(1):334-350. https://doi.org/10.1038/s44319-023-00003-7.

Southwick Associates. 2018. Hunting in America. An Economic Force for Conservation. https://www.fishwildlife.org/application/files/3815/3719/7536/Southwick_Assoc_-_NSSF_Hunting_Econ.pdf.

Spickler, A.R. 2016. Scrapie. Retrieved from http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php.

Spraker, T.R., M.W. Miller, E.S. Williams, D.M. Getzy, W.J. Adrian, G.G. Schoonveld, R.A. Spowart, K.I. O'Rourke, J.M. Miller, and P.A. Merz. 1997. Spongiform encephalopathy in free-ranging mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus) and Rocky Mountain elk (Cervus elaphus nelsoni) in northcentral Colorado. *Journal of Wildlife Diseases* 33(1):1-6. https://doi.org/10.7589/0090-3558-33.1.1.

Spraker, T.R., R.R. Zink, B.A. Cummings, M.A. Wild, M.W. Miller, and K.I. O'Rourke. 2002a. Comparison of histological lesions and immunohistochemical staining of proteinase-resistant prion protein in a naturally occurring spongiform encephalopathy of free-ranging mule deer (Odocoileus hemionus) with those of chronic wasting disease of captive mule deer. *Veterinary Pathology* 39(1):110-9. https://doi.org/10.1354/vp.39-1-110.

Spraker, T.R., K.I. O'Rourke, A. Balachandran, R.R. Zink, B.A. Cummings, M.W. Miller, and B.E. Powers. 2002b. Validation of monoclonal antibody F99/97.6.1 for immunohistochemical staining of brain and tonsil in mule deer (Odocoileus hemionus) with chronic wasting disease. *Journal of Veterinary Diagnostic Investigation* 14(1):3-7. https://doi.org/10.1177/104063870201400102.

Spraker, T.R., A. Balachandran, D. Zhuang, and K.I. O'Rourke. 2004. Variable patterns of distribution of PrP(CWD) in the obex and cranial lymphoid tissues of Rocky Mountain elk (Cervus elaphus nelsoni) with subclinical chronic wasting disease. *Veterinary Record* 155(10):295-302. https://doi.org/10.1136/vr.155.10.295.

Spraker, T.R., K.I. O'Rourke, T. Gidlewski, J.G. Powers, J.J. Greenlee, and M.A. Wild. 2010. Detection of the abnormal isoform of the prion protein associated with chronic wasting disease in the optic pathways of the brain and retina of Rocky Mountain elk (Cervus elaphus nelsoni). *Veterinary Pathology* 47(3):536-46. https://doi.org/10.1177/0300985810363702.

Spraker, T.R., T. Gidlewski, J.G. Powers, T. Nichols, A. Balachandran, B. Cummings, M.A. Wild, K. VerCauteren, and K.I. O'Rourke. Progressive accumulation of the abnormal conformer of the prion protein and spongiform encephalopathy in the obex of nonsymptomatic Rocky Mountain elk (Cervus elaphus nelsoni) with chronic wasting disease. *Journal of Veterinary Diagnostic Investigation* 27(4):431-441. https://doi.org/10.1177/1040638715593368.

Spraker, T.R., T. Gidlewski, J.G. Powers, T.A. Nichols, and M.A. Wild. 2023. Distribution of the misfolded isoform of the prion protein in peripheral tissues and spinal cord of Rocky Mountain elk (Cervus elaphus nelsoni) with naturally occurring chronic wasting disease. *Veterinary Clinical Pathology* 60(4):420-433. https://doi.org/10.1177/03009858231173467.

Stafford, N.T., M.D. Needham, J.J. Vaske, and J. Petchenik. 2007. Hunter and Nonhunter beliefs about Chronic Wasting Disease in Wisconsin. *The Journal of Wildlife Management* 71(5):1739-1744.

Stasiak, I., T. Perry, and T. Bollinger. 2023. Indications of population impacts and declining hunter participation in the South Saskatchewan River Valley: A growing management concern. 4th International Chronic Wasting Disease Symposium.

Stankey, G.H., R.N. Clark, and B.T. Bormann. 2005. *Adaptive management of natural resources: theory, concepts, and management institutions. Gen. Tech. Rep. PNW-GTR-654*. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. https://www.fs.usda.gov/pnw/pubs/pnw_gtr654.pdf.

Stear, M.J., G. Nikbakht, L. Matthews, and N.N. Jonsson. 2012. Breeding for disease resistance in livestock and fish. *CABI Reviews* 1–10. https://doi.org/10.1079/pavsnnr20127007.

Stephen, C. 2022. "An Emerging Disease Agenda for Wildlife Health Management." In *Wildlife Population Health*, edited by C. Stephen, 169-176. Cham, Switzerland: Springer Nature Switzerland AG.

Stephen, C. 2022. "Human Dimensions of Wildlife Health Management." In *Wildlife Population Health*, edited by C. Stephen, 205-210. Cham: Springer International Publishing.

Storm, D.J., M.D. Samuel, R.E. Rolley, P. Shelton, N.S. Keuler, B.J. Richards, and T.R. Van Deelen. 2013. Deer density and disease prevalence influence transmission of chronic wasting disease in white-tailed deer. *Ecosphere* 4(1):art10. https://doi.org/10.1890/ES12-00141.1.

Stubier, P., C. Hill, B. Monty, Z Ramirez, and M. Regan. 2006. An Evaluation: Chronic Wasting Disease, Department of Natural Resources. Report 06-13. Report prepared by the Legislative Audit Committee for the Joint Legislative Audic Committee, Madison, WI. https://www.drdeer.com/uploads/cms/nav-17-58012043e2df1.pdf, Accessed November 13, 2024.

Tamgüney, G., M.W. Miller, L.L. Wolfe, T.M Sirochman, D.V. Glidden, C. Palmer, A. Lemus, S.J. DeArmond, and S.B. Prusiner. 2009. Asymptomatic deer excrete infections prions in faeces. *Nature* 461:529-532. https://doi.org/10.1038/nature08289.

Taschuk, R., K. Marciniuk, P. Määttänen, C. Madampage, P. Hedlin, A. Potter, J. Lee, N. R. Cashman, P. J. Griebel, and S. Napper. 2014. Safety, specificity and immunogenicity of a PrP(Sc)-specific prion vaccine based on the YYR disease specific epitope. *Prion* 8(1):51-9. https://doi.org/10.4161/pri.27962.

Tewari, D., M. Fasnacht, M. Ritzman, J. Livengood, J. Bower, A. Lehmkuhl, T. Nichols, A. Hamberg, K. Brightbill, and D. Henderson. 2022. Detection of chronic wasting disease in feces and recto-anal mucosal associated lymphoid tissues with RT-QuIC in a naturally infected farmed white-tailed deer herd. *Frontiers in Veterinary Science* 9:959555. https://doi.org/10.3389/fvets.2022.959555.

Thackray, A.M., E.E. McNulty, A.V. Nalls, A. Smith, E. Comoy, G. Telling, S.L. Benestad, O. Andréoletti, C.K. Mathiason, and R. Bujdoso. 2024. Lack of prion transmission barrier in human PrP transgenic Drosophila. *Journal of Biological Chemistry* 107617. https://doi.org/10.1016/j.jbc.2024.107617.

Thapa, S., C. Marrero Winkens, W. Tahir, M.I. Arifin, S. Gilch, and H.M. Schatzl. 2022. Gene-Edited Cell Models to Study Chronic Wasting Disease. *Viruses* 14(3). https://doi.org/10.3390/v14030609.

The United States Congress. 2020. Text - H.R.877 - 116th Congress (2019-2020): Modernizing the Pittman-Robertson Fund for Tomorrow's Needs Act.

The Manhattan Principles. 2004. Building Interdisciplinary Bridges to Health in a "Globalized World", Rockefeller University, New York City.

Thompson, A.K., M.D. Samuel, and T.R. Van Deelen. 2008. Alternative Feeding Strategies and Potential Disease Transmission in Wisconsin White-Tailed Deer. *The Journal of Wildlife Management* 72(2):416-421. https://doi.org/10.2193/2006-543.

Thompson, N.E., and J.R. Mason. 2022. "The cost of combatting chronic wasting disease." *The Wildlife Professional* 15:46-49. https://www.fishwildlife.org/application/files/6416/6879/5372/Thompson_Mason-CWD_Costs-V2-Clean-29Aug22.pdf.

Thompson, N.E., M.H.J. Huang, S.A. Christensen, and S. Demarais. 2023. Wildlife agency responses to chronic wasting disease in free-ranging cervids. *Wildlife Society Bulletin* 47(2):e1435. https://doi.org/10.1002/wsb.1435.

Thompson, N., D. Butts, M. Murillo, D. O'Brien, S. Christensen, W. Porter, and G. Roloff. 2024. An individual-based model for direct and indirect transmission of chronic wasting disease in free-ranging white-tailed deer. *Ecological Modelling* 491:110697. https://doi.org/10.1016/j.ecolmodel.2024.110697.

Thomsen, B.V., D.A. Schneider, K.I. O'Rourke, T. Gidlewski, J. McLane, R.W. Allen, A.A. McIsaac, G.B. Mitchell, D.P. Keane, T.R. Spraker, and A. Balachandran. 2012. Diagnostic accuracy of rectal mucosa biopsy testing for chronic wasting disease within white-tailed deer (Odocoileus virginianus) herds in North America: effects of age, sex, polymorphism at PRNP codon 96, and disease progression. *Journal of Veterinary Diagnostic Investigation* 24(5):878-87. https://doi.org/10.1177/1040638712453582.

Towne, E.G. 2000. Prairie vegetation and soil nutrient responses to ungulate carcasses. *Oecologia* 122(2):232-239. https://doi.org/10.1007/pl00008851.

Tranulis, M.A., D. Gavier-Widén, J. Våge, M. Nöremark, S.L. Korpenfelt, M. Hautaniemi, L. Pirisinu, R. Nonno, and S.L. Benestad. 2021. Chronic wasting disease in Europe: new strains on the horizon. *Acta Veterinaria Scandinavica* 63(1):48. https://doi.org/10.1186/s13028-021-00606-x.

Tranulis, M.A., and M. Tryland. 2023. The Zoonotic Potnetial of Chronic Wasting Disease – A Review. *Foods* 12(4):824. https://doi.org/10.3390/foods12040824.

Trout, J., M. Roberts, M. Tabet, E. Kotkowski, and S. Horn. 2024. Two Hunters from the Same Lodge Afflicted with Sporadic CJD: Is Chronic Wasting Disease to Blame? (P7-13.002). *Neurology* 102 (17_supplement_1):216. https://doi.org/doi:10.1212/WNL.0000000000204407.

U.S. Department of the Interior, Bureau of Indian Affairs, Department of Health and Human Services, and Indian Health Service. 1996. Indian Self Determination and Education Assistance Act.

Uehlinger, F.D., A.C. Johnston, T.K. Bollinger, and C.L. Waldner. 2016. Systematic review of management strategies to control chronic wasting disease in wild deer populations in North America. *BMC Veterinary Research* 12(1):173. https://doi.org/10.1186/s12917-016-0804-7.

Ufer, D.J., S.A. Christensen, D.L. Ortega, N. Pinizzotto, and K. Schuler. 2022. Stamping out wildlife disease: Are hunter-funded stamp programs a viable option for chronic wasting disease management? *Conservation Science and Practice* 4(9):e12779. https://doi.org/10.1111/csp2.12779.

Ufer, D.J., S.A. Christensen, E. Pomeranz, and D.L. Ortega. 2023. A behavioral economic assessment of the role of stakeholder preferences in managing an infectious wildlife disease. *Wildlife Society Bulletin* 47(2):e1411. https://doi.org/10.1002/wsb.1411.

United States 116th Congress. 2020. America's Conservation Enhancement Act. P.L. 116–188.

United States Congress. 2021. H.R.5608 - 117th Congress (2021-2022): Chronic Wasting Disease Research and Management Act.

United States Livestock Sanitary Association. 1959. Sixty-Third Annual Meeting of the United States Livestock Sanitary Association. San Francisco, California.

United States Livestock Sanitary Association. 1961. Sixty-Fifth Annual Meeting of the United States Livestock Sanitary Association. Minneapolis, Minnesota.

United States Livestock Sanitary Association. 1971. Seventy-Fifth Annual Meeting of the United States Livestock Sanitary Association. Oklahoma City, Oklahoma.

Urayama, A., R. Morales, M.L. Niehoff, W.A. Banks, and C. Soto. 2011. Initial fate of prions upon peripheral infection: half-life, distribution, clearance, and tissue uptake. *The FASEB Journal* 25(8):2792-2803. https://doi.org/10.1096/fj.11-180729.

USDA. 2019. Specified Risk Material (SRM) Control. https://www.fsis.usda.gov/sites/default/files/media_file/2021-11/06b_IM_SRM-control-02262019.pdf.

USDA Animal and Plant Health Inspection Service. 2005. Bovine Tuberculosis Eradication. Department of Agriculture and Animal Plant Health Inspection Service. https://www.aphis.usda.gov/sites/default/files/tb-umr.pdf.

USDA Animal and Plant Health Inspection Service. 2006. Bovine Spongiform Encephalopathy (BSE) Ongoing Surveillance Plan. https://www.aphis.usda.gov/sites/default/files/BSE_ongoing_surv_plan_final.pdf (accessed August 28, 2024).

USDA Animal and Plant Health Inspection Service. 2019. Chronic Wasting Disease Program Standards. https://www.aphis.usda.gov/sites/default/files/cwd-program-standards.pdf (accessed August 24, 2024).

USDA Animal and Plant Health Inspection Service. 2020. Prevention and Control of H5 and H7 Avian Influenza in the Live Bird Marketing System Uniform Standards for a State Federal-Industry Cooperative Program. https://www.aphis.usda.gov/media/document/309/file.

USDA Animal and Plant Health Inspection Service, 2021. USDA APHIS Tribal Nations Wild Cervid Chronic Wasting Disease Opportunities 2021 Cooperative Agreements. 2021 Project Executive Summaries. https://www.aphis.usda.gov/sites/default/files/cwd-funding-ws-tribal-executive-summaries.pdf.

USDA Animal and Plant Health Inspection Service. 2023. National Scrapie Eradication Program Fiscal Year 2022 Report: October 1, 2021, to September 30, 2022. Strategy and Policy Unit Animal and Plant Health Inspection Service-Veterinary Services, Sheep and Goat Health Center U.S. Department of Agriculture. https://www.aphis.usda.gov/sites/default/files/scrapie-annual-report.pdf.

USDA Animal and Plant Health Inspection Service. 2024. Farmed Cervid Chronic Wasting Disease - 2024 Cooperative Agreement Funding Opportunity Frequently Asked Questions (FAQs). https://www.aphis.usda.gov/sites/default/files/fy24-vs-cwd-faq.pdf (accessed July 25, 2024).

USDA National Scrapie Surveillance Plan. 2022. Animal and Plant Health Inspection Service, United States Department of Agriculture. https://www.aphis.usda.gov/sites/default/files/national_scrapie_surv_plan.pdf.

USAHA Committee on Farmed Cervidae, Committee on Poultry and other Avian Species, and Committee on Animal Emergency Management. 2024. Increased Funding for Research and Operations at the United States Department of Agriculture, Agricultural Research Services National Animal Disease Center, 2024 Resolution. 128th Annual Meeting. https://usaha.org/wp-content/uploads/2024/10/2024-USAHA-Resolutions-ALL.pdf (accessed November 8, 2024).

USAHA Committee on Wildlife Diseases. 2000. *Report of the Committee on Wildlife Diseases.*

USAHA. 1998. Proceedings of the One Hundred and Second Annual Meeting of the United States Animal Health Association. Richmond, VA: Pat Cambell & Associates; and Carter Printing Company. https://usaha.org/upload/Proceedings/1998_ONE_HUNDRED_AND_SECOND_ANNU.pdf (accessed July 25, 2024).

USAHA. 2001. Proceedings of the One Hundred and Fifth Annual Meeting of the United States Animal Health Association. Richmond, VA: Pat Cambell & Associates. https://usaha.org/upload/Proceedings/2001_ONE_HUNDRED_AND_FIFTH_ANNUA.pdf (accessed July 25, 2024).

USGS National Wildlife Health Center. 2024. "Distribution of Chronic Wasting Disease in North America." https://www.usgs.gov/media/images/distribution-chronic-wasting-disease-north-america-0 (accessed July 25, 2024).

Vander Wal, E., D. Garant, S. Calmé. C.A. Chapman, M. Festa-Bianchet, V.Millien, S. Rioux-Paquette, and F. Pelletier. 2014. Applying evolutionary concepts to wildlife disease ecology and management. *Evolutionary Applications* 7(7):856-868. https://doi.org/10.1111/eva.12168.

van Keulen, L.J., M.E. Vromans, and F.G. van Zijderveld. 2002. Early and late pathogenesis of natural scrapie infection in sheep. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 110(1):23-32. https://doi.org/10.1034/j.1600-0463.2002.100104.x.

van Keulen, L.J., A. Bossers, and F. van Zijderveld. 2008. TSE pathogenesis in cattle and sheep. *Veterinary Research*. 39 (4): 24. https://doi.org/10.1051/vetres:2007061.

Vaske, J.J. 2010. Lessons Learned from Human Dimensions of Chronic Wasting Disease Research. Human Dimensions of Wildlife. 15 (3): 165-179. https://doi.org/10.1080/10871201003775052.

Vaske, J.J., and K.M. Lyon. 2011. CWD prevalence, perceived human health risks, and state influences on deer hunting participation. *Risk Analysis* 31(3):488-96. https://doi.org/10.1111/j.1539-6924.2010.01514.x.

Vaske, J.J., M.D. Needham, N.T. Stafford, K. Green, and J. Petchenik. 2006. Information Sources and Knowledge about Chronic Wasting Disease in Colorado and Wisconsin. *Human Dimensions of Wildlife* 11:191-202. https://doi.org/10.1080/10871200600669981.

Vaughan Branch, J., J. Karlen, J. Organ, C. Bishop, M. Mitchell, R. Regan, and J. Millspaugh. 2022. Echoes of 1937: Recovering America's Wildlife Act would bring wildlife conservation funding full circle. *Conservation Letters* 15. https://doi.org/10.1111/conl.12890.

Vaughn, John B., Jr., Phyllis Gerhardt, and Kenneth W. Newell. 1965. Excretion of Street Rabies Virus in the Saliva of Dogs. *JAMA* 193(5):363-368. https://doi.org/10.1001/jama.1965.03090050039010.

VerCauteren, K.C., J.L. Pilon, P.B. Nash, G.E. Phillips, and J.W. Fischer. 2012. Prion Remains Infectious after Passage through Digestive System of American Crows (Corvus brachyrhynchos). *PLOS ONE* 7(10):e45774. https://doi.org/10.1371/journal.pone.0045774.

Velásquez, C.D., C. Kim, A. Herbst, N. Daude, M.C. Garza, H. Wille, J. Aiken, and D. McKenzie. 2015. Deep Prion Proteins Modulate the Emergence and Adaptation of Chronic Wasting Disease. *Journal of Virology* 89(24). https://doi.org/10.1128/jvi.02010-15.

Vikøren, T., J. Våge, K.I. Madslien, K.H. Røed, C.M. Rolandsen, L. Tran, P. Hopp, V. Veiberg, M. Heum, T. Moldal, C.G.D. Neves, K. Handeland, B. Ytrehus, Ø. Kolbjørnsen, H. Wisløff, R. Terland, B. Saure, K.M. Dessen, S.G. Svendsen, B.S. Nordvik, and S.L. Benestad. 2019. First Detection of Chronic Wasting Disease in a Wild Red Deer (Cervus elaphus) in Europe. *Journal of Wildlife Diseases* 55(4):970-972. https://doi.org/10.7589/2018-10-262.

Waddell, L., J. Greig, M. Mascarenhas, A. Otten, T. Corrin, and K. Hierlihy. 2017. Current evidence on the transmissibility of chronic wasting disease prions to humans – A systematic review. *Transboundary and Emerging Diseases* 65(1):37-49. https://doi.org/10.1111/tbed.12612.

Walsh, D.P., and M.W. Miller. 2010. A weighted surveillance approach for detecting chronic wasting disease foci. *Journal of Wildlife Diseases*. 46 (1): 118-35. https://doi.org/10.7589/0090-3558-46.1.118.

Walsh, D.P. 2012. Enhanced surveillance strategies for detecting and monitoring chronic wasting disease in free-ranging cervids: U.S. Geological Survey Open-File Report 2012–1036.

Walsh, T. 2018. Fuzzy gold standards: Approaches to handling an imperfect reference standard. *Journal of Dentistry* 74:S47-S49. https://doi.org/10.1016/j.jdent.2018.04.022.

Walter, W.D., D.P. Walsh, M.L. Farnsworth, D.L. Winkelman, and M.W. Miller. 2011. Soil clay content underlies prion infection odds. *Nature Communications* 2:200. https://doi.org/10.1038/ncomms1203.

Wang, F., X. Wang, C.G. Yuan, and J. Ma. 2010. Generating a prion with bacterially expressed recombinant prion protein. *Science* 327(5969):1132-5. https://doi.org/10.1126/science.1183748.

Wang, Z., K. Qin, M.V. Camacho, I. Cali, J. Yuan, P. Shen, J. Greenlee, Q. Kong, J.A. Mastrianni, and W.Q. Zou. 2021. Generation of human chronic wasting disease in transgenic mice. *Acta Neuropathologica Communications.* 9 (1): 158. https://doi.org/10.1186/s40478-021-01262-y.

Wasmer, C., A. Lange, H. Van Melckebeke, A.B. Siemer, R. Riek, and B.H. Meier. 2008. Amyloid Fibrils of the HET-s(218–289) Prion Form a β Solenoid with a Triangular Hydrophobic Core. *Science* 319(5869):1523-1526. https://doi.org/doi:10.1126/science.1151839.

Wasserberg, G., E.E. Osnas, R.E. Rolley, and M.D. Samuel. 2009. Host culling as an adaptive management tool for chronic wasting disease in white-tailed deer: a modelling study. *Journal of Applied Ecology*. 46 (2): 457-466. https://doi.org/10.1111/j.1365-2664.2008.01576.x.

Wells, G.A., A.C. Scott, C.T. Johnson, R.F. Gunning, R.D. Hancock, M. Jeffrey, M. Dawson, and R. Bradley. 1987. A novel progressive spongiform encephalopathy in cattle. *Veterinary Record* 121(18):419-20. https://doi.org/10.1136/vr.121.18.419.

Western Association of Fish and Wildlife Agencies. 2017. Recommendations for Adaptive Management of Chronic Wasting Disease in the West. WAFWA Wildlife Health Committee and Mule Deer Working Group. Edmonton, Alberta, Canada and Fort Collins, Colorado.

Wild, M.A., T.R. Spraker, C.J. Sigurdson, K.I. O'Rourke, and M.W. Miller. 2002. Preclinical diagnosis of chronic wasting disease in captive mule deer (Odocoileus hemionus) and white-tailed deer (Odocoileus virginianus) using tonsillar biopsy. *Journal of General Virology* 83(10):2629-2634. https://doi.org/10.1099/0022-1317-83-10-2629.

Wild, M.A., N.T. Hobbs, M.S. Graham, and M.W. Miller. 2011. The role of predation in disease control: a comparison of selective and nonselective removal on prion disease dynamics in deer. *Journal of Wildlife Diseases* 47(1):78-93. https://doi.org/10.7589/0090-3558-47.1.78.

Wilesmith, J.W., G.A. Wells, M.P. Cranwell, and J.B. Ryan. 1988. Bovine spongiform encephalopathy: epidemiological studies. *Veterinary Record* 123(25):638-44.

Wilesmith, J.W., J.B.M. Ryan, M.A. Stevenson, R.S. Morris, D.U. Pfeiffer, D. Lin, R. Jackson, and R.L. Sanson. 2000. Temporal aspects of the epidemic of bovine spongiform encephalopathy in Great Britain: holding.associated risk factors for the disease. *Veterinary Record* 147(12):319-325. https://doi.org/10.1136/vr.147.12.319.

Will, R.G., J.W. Ironside, M. Zeidler, S.N. Cousens, K. Estibeiro, A. Alperovitch, S. Poser, M. Pocchiari, A. Hofman, and P.G. Smith. 1996. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 347(9006):921-5. https://doi.org/10.1016/s0140-6736(96)91412-9.

Williams, B.K., and E.D. Brown. 2013. Adaptive Management: From More Talk to Real Action. *Environmental Management* 53:465-479.

Williams, E. S. 2005. Chronic wasting disease. *Vet Pathol* 42(5):530-49. https://doi.org/10.1354/vp.42-5-530.

Williams, E.S., and S. Young. 1980. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. *Journal of Wildlife Diseases* 16(1):89-98. https://doi.org/10.7589/0090-3558-16.1.89.

Williams, E.S., and S. Young. 1992. Spongiform encephalopathies in Cervidae. *Revue Scientifique et Technique*. 11 (2): 551-67. https://doi.org/10.20506/rst.11.2.611.

Williams, E.S., and S. Young. 1993. Neuropathology of chronic wasting disease of mule deer (Odocoileus hemionus) and elk (Cervus elaphus nelsoni). *Veterinary Clinical Pathology* 30(1):36-45. https://doi.org/10.1177/030098589303000105.

Williams, E.S., and M.W. Miller. 2002. Chronic wasting disease in deer and elk in North America. *Revue Scientifique et Technique*. 21 (2): 305-16. https://doi.org/10.20506/rst.21.2.1340.

Williams, E.S. 2005. Chronic wasting disease. *Veterinary Clinical Pathology* 42(5):530-49. https://doi.org/10.1354/vp.42-5-530.

Williams, E.S., M.W. Miller, T.J. Kreeger, R.H. Kahn, and R.T. Thorne. 2002. Chronic Wasting Disease of Deer and Elk: A Review with Recommendations for Management. *The Journal of Wildlife Management* 66(3):551-563.

Williams, E.S., and M.W. Miller. 2003. Transmissible spongiform encephalopathies in non-domestic animals: origin, transmission and risk factors. *Revue Scientifique et Technique* 22(1):145-56. https://doi.org/10.20506/rst.22.1.1385.

Williams, A.L., T.J. Kreeger, and B.A. Schumaker. 2014. Chronic wasting disease model of genetic selection favoring prolonged survival in Rocky Mountain elk (Cervus elaphus). *Ecosphere* 5(5):art60. https://doi.org/10.1890/ES14-00013.1.

Williams, E.S., D. O'Toole, M.W. Miller, T.J. Kreeger, and J.E. Jewell. 2018. Cattle (Bos Taurus) Resist Chronic Wasting Disease Following Oral Inoculation Challenge or Ten Years' Natural Exposure in Contaminated Environments. *Journal of Wildlife Diseases* 54(3):460-470. https://doi.org/10.7589/2017-12-299.

Wilson, G.A., S.M. Nakada, T.K. Bollinger, M.J. Pybus, E.H. Merrill, and D.W. Coltman. 2009. Polymorphisms at the PRNP gene influence susceptibility to chronic wasting disease in two species of deer (Odocoileus Spp.) in western Canada. *Journal of Toxicology and Environmental Health* 72. A(17-18):1025-9. https://doi.org/10.1080/15287390903084264.

Wilson, R., C. Plinston, N. Hunter, C. Casalone, C. Corona, F. Tagliavini, S. Suardi, M. Ruggerone, F. Moda, S. Graziano, M. Sbriccoli, F. Cardone, M. Pocchiari, L. Ingrosso, T. Baron, J. Richt, O. Andreoletti, M. Simmons, R. Lockey, J.C. Manson, and R.M. Barron. 2012. Chronic wasting disease and atypical forms of bovine spongiform encephalopathy and scrapie are not transmissible to mice expressing wild-type levels of human prion protein. *Journal of General Virology* 93(7):1624-1629. https://doi.org/10.1099/vir.0.042507-0.

Wineland, N.E. 1993. Epidemiology of reported scrapie in the United States: 1947-1991. Master of Science, Clinical Sciences Department, Colorado State University.

Winter, S.N., and L.E. Escobar. 2020. Chronic Wasting Disease Modeling: An Overview. *Journal of Wildlife Diseases* 56(4):741-758. https://doi.org/10.7589/2019-08-213.

Wobeser, G.A. 2007. *Diseases in Wild Animals: Investigation and Management*. 2nd ed. Berlin Heidelberg, Germany: Springer-Verlag.

Woodgate, S.L., and R.G. Wilkinson. 2021. The role of rendering in relation to the bovine spongiform encephalopathy epidemic, the development of EU animal by-product legislation and the reintroduction of rendered products into animal feeds. *Annals of Applied Biology* 178:430-441. https://doi.org/10.1111/aab.12676.

World Organization for Animal Health (WOAH). 2023. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Twelfth Edition.

Wolfe, L.L., M.W. Miller, and E. Williams. 2004. Feasibility of "Test-and-Cull" for Managing Chronic Wasting Disease in Urban Mule Deer. *Wildlife Society Bulletin (1973-2006)* 32(2):500-505.

Wolfe, L.L., D.A. Kocisko, B. Caughey, and M.W. Miller. 2012. Assessment of prospective preventive therapies for chronic wasting disease in mule deer. *Journal of Wildlife Diseases* 48(2):530-3. https://doi.org/10.7589/0090-3558-48.2.530.

Wolfe, L.L., K.A. Fox, and M.W. Miller. 2014. "Atypical" chronic wasting disease in PRNP genotype 225FF mule deer. *Journal of Wildlife Diseases*. 50 (3): 660-5. https://doi.org/10.7589/2013-10-274.

Wolfe, L.L., M.K. Watry, M.A. Sirochman, T.M. Sirochman, and M.W. Miller. 2018. Evaluation of a Test and Cull Strategy for Reducing Prevalence of Chronic Wasting Disease in Mule Deer (Odocoileus hemionus). *Journal of Wildlife Diseases* 54(3):511-519. https://doi.org/10.7589/2018-01-015.

Wolfe, L.L., K.A. Fox, K.A. Griffin, and M.W. Miller. 2022. Mountain Lions (Puma concolor) Resist Long-term Dietary Exposure to Chronic Wasting Disease. *Journal of Wildlife Diseases* 58(1):40-49. https://doi.org/10.7589/jwd-d-21-00029.

Wood, M.E., P. Griebel, M.L. Huizenga, S. Lockwood, C. Hansen, A. Potter, N. Cashman, J.W. Mapletoft, and S. Napper. 2018. Accelerated onset of chronic wasting disease in elk (Cervus canadensis) vaccinated with a PrP(Sc)-specific vaccine and housed in a prion contaminated environment. *Vaccine* 36(50):7737-7743. https://doi.org/10.1016/j.vaccine.2018.10.057.

Wyckoff, A.C., N. Galloway, C. Meyerett-Reid, J. Powers, T. Spraker, R. J. Monello, B. Pulford, M. Wild, M. Antolin, K. VerCauteren, and M. Zabel. 2015. Prion amplification and hierarchical Bayesian modeling refine detection of prion infection. *Scientific Reports* 5:8358. https://doi.org/10.1038/srep08358.

Wyckoff, A.C., S. Kane, K. Lockwood, J. Seligman, B. Michel, D. Hill, A. Ortega, M.R. Mangalea, G.C. Telling, M.W. Miller, K. Vercauteren, and M.D. Zabel. 2016. Clay Components in Soil Dictate Environmental Stability and Bioavailability of Cervid Prions in Mice. *Frontiers in Microbiology* 7. https://doi.org/10.3389/fmicb.2016.01885.

Xie, Lusi, Wiktor Adamowicz, and Patrick Lloyd-Smith. 2023. Spatial and temporal responses to incentives: An application to wildlife disease management. *Journal of Environmental Economics and Management* 117:102752. https://doi.org/10.1016/j.jeem.2022.102752.

Xu, J., E.H. Merrill, and M.A. Lewis. 2022. Spreading speed of chronic wasting disease across deer groups with overlapping home ranges. *Journal of Theoretical Biology* 547:111135. https://doi.org/10.1016/j.jtbi.2022.111135.

Yang, J., and E. Goddard. 2011. Canadian Consumer Responses to BSE with Heterogeneous Risk Perceptions and Risk Attitudes. *Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie* 59(4):493-518. https://doi.org/10.1111/j.1744-7976.2011.01225.x.

Yekutiel, P. 1980. Eradication of infectious diseases: a critical study. In *Contributions to epidemiology and biostatistics, Volume 2.* S. Karger: Basel, Switzerland.

Yoder, C., Wolf, T., Schwabenlander, M., Yustinyuk, V. 2023. Chronic Wasting Disease surveillance in Minnesota Indian Country, development of a regional surveillance system to protect tribal subsistence species.

Yu, G., J. Chen, Y. Xu, C. Zhu, H. Yu, S. Liu, H. Sha, J. Chen, X. Xu, Y. Wu, A. Zhang, J. Ma, and G. Cheng. 2009. Generation of goats lacking prion protein. *Molecular Reproduction & Development* 76(1):3. https://doi.org/10.1002/mrd.20960.

Yuan, Q., G. Rowden, T.M. Wolf, M.D. Schwabenlander, P.A. Larsen, S.L. Bartelt-Hunt, and J.C. Bartz. 2022. Sensitive detection of chronic wasting disease prions recovered from environmentally relevant surfaces. *Environment International* 166:107347. https://doi.org/10.1016/j.envint.2022.107347.

Zabel, M.D., and A.C. Avery. 2015. Prions–not your immunologist's pathogen. *PLOS Pathogen* 11(2):e1004624. https://doi.org/10.1371/journal.ppat.1004624.

Zabel, M., and A. Ortega. 2017. The Ecology of Prions. *Microbiology and Molecular Biology Reviews* 81(3). https://doi.org/doi:10.1128/mmbr.00001-17.

Zimmer, N.M.P., P.C. Boxall, and W.L. Adamowicz. 2012. The Impacts of Chronic Wasting Disease and its Management on Recreational Hunters. *Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie* 60(1):71-92. https://doi.org/https://doi.org/10.1111/j.1744-7976.2011.01232.x.

Appendix A
Committee Biographical Information

Lonnie King (*Chair*) is an Academy Professor and Dean Emeritus in the College of Veterinary Medicine at the Ohio State University (OSU). He previously served as OSU's Vice-President for Agriculture and was the first Director for the National Center for Zoonotic, Vector-borne, and Enteric Diseases at the Centers for Disease Control and Prevention and the administrator of the U.S. Department of Agriculture's Animal Plant Health Inspection Service (APHIS) where he also was the nation's Chief Veterinary Officer for five years. King's research interests and expertise include emerging infectious diseases, zoonotic diseases, antimicrobial resistance, food safety and security, global health, One Health, and leadership development. He received an honorary degree from Tufts University in 2022, was awarded the Global One Health Award from the World Veterinary Medical Association, and the Meritorious Award from the World Organization for Animal Health. King received a B.S. and DVM from OSU, an M.S. in epidemiology from the University of Minnesota, and an MPA from American University. He is boarded in the American College of Veterinary Preventive Medicine and completed the Senior Executive Fellowship in leadership from Harvard University. He has served on numerous National Academy consensus study committees as well as the Forum on Microbial Threats and the One Health Action Collaborative.

Sonja Ann Christensen is an assistant professor in the Department of Fisheries and Wildlife at Michigan State University. Christensen has worked in the field of wildlife research and management for 20 years and multiple state agencies and universities. From April 2008 – August 2011, she worked for the Massachusetts Division of Fisheries and Wildlife as a deer and moose project leader with statewide wildlife management responsibilities, including supervising the statewide cervid disease program, prior to returning to academia and obtaining her PhD in disease ecology. She serves as the Principal Investigator for the Christensen Lab for Wildlife Population Health where her and her students research uses quantitative modeling, field ecology, and social science methods to study wildlife disease ecology, population ecology, and wildlife management, with a special focus on diseases associated with cervid species. Christensen's outreach program includes working with the Association of Fish and Wildlife Agencies to provide technical support and coordination around fish and wildlife health issues and supporting capacity building for wildlife health. She is a co-founder of the CWD Research Consortium and multistate project (NC1209). She also serves as an uncompensated member of the USGS Biological Threats and Invasive Species Research Program Council where she provides state agency perspectives for research programs for that group. Christensen received an M.S. in fisheries and wildlife science from the Pennsylvania State University and a Ph.D. in fisheries and wildlife with a specialization in disease ecology and conservation medicine from Michigan State University. She is a past President of the Michigan Chapter of The Wildlife Society and previous board member of The Wildlife Society's Wildlife Disease Working Group. She is a member of the Wildlife Disease Association and the Native American Fish and Wildlife Society. Christensen has received compensation for service as the Fish and Wildlife Health Coordinator from the Association of Fish and Wildlife Agencies. Christensen provides interviews for media and podcasts, and outreach for state fish and wildlife agencies regarding CWD and other cervid diseases.

Matthew C. Dunfee is the Director of Special Programs for the Wildlife Management Institute (WMI) and served as the Conservation Program Specialist in WMI's Headquarters working on projects related to North American wildlife conservation, private lands habitat programs, and free-ranging cervid disease management. He also serves as the Director of the Chronic Wasting Disease (CWD) Alliance, the Chair

of the North American Wildlife and Natural Resources Conference, Co-Chair of the National Hunting and Shooting Sports Action Plan, Chair of the CWD Applied Research Management Grant Program, and Administrator for the International CWD Information and Data Sharing Hub. Dunfee serves on numerous professional committees and boards including the Association of Fish and Wildlife Agencies' (AFWA) Fish and Wildlife Health Committee. He has led state, regional, and national strategic decision-making processes targeting effective management of CWD in wild cervid populations, including in Colorado, Tennessee, Kansas, and North Dakota, and the USGS. He was awarded the IHEA-USA's Dr. Edward Kozicky Award and a Distinguished Service award from the Council to Advance Hunting and the Shooting Sports. Dunfee received a B.S. in fish, wildlife, and conservation biology from Colorado State University. Dunfee served as a science and technical advisor for several conservation non-governmental organizations within the past five years, including, for example, the National Wildlife Federation, the Boone and Crockett Club, and the National Military Fish and Wildlife Association. Dunfee has provided talks and interviews for numerous podcasts, organizations, and media outlets on the history, status, and available CWD management tools.

David Finnoff is a Wyoming Excellence Chair and Professor of Economics at the University of Wyoming. His research focus is on risk management in coupled human, natural systems. He has led many research projects that include management of grizzly bears and wolves, optimal endangered species listing decisions, management of wild game species facing threats of brucellosis and chronic wasting disease (CWD), and on management of forests under threat of beetle outbreaks. He has been awarded federal grants to conduct research, including projects aimed at managing coupled systems facing tipping points, the development integrated economic/epidemiological models for management of infectious diseases that threaten humans, and for the development of linked economic/ecosystem models. Other funded projects include several aimed at the development of integrated models for management of economic and ecological systems subject to the risk of invasive species. Recently, Finnoff has extended a line of work on optimal social investments to reduce pandemic risks to consider the economic and social effects of the COVID-19 pandemic, the role of public policy in reducing the risks associated with COVID-19, and on developing modeling frameworks to consider the implications of future novel disease risks that incorporate risk driven behavioral responses and how concern for others influences decisions. Finnoff received a B.S. and Ph.D. in economics from the University of Wyoming. Finnoff is a co-author on a paper in which a predictive model simulating animal welfare related to CWD transmission in a variety of wildlife management scenarios related to elk feedgrounds is described.

Thomas Gidlewski retired from the USDA, Animal Plant Health Inspection Service (APHIS) in 2021 after 35 years of service. He is currently an instructor for the APHIS foreign animal disease courses at the Plum Island Animal Disease Center in New York. Prior to retirement from USDA APHIS, he was the Program Manager for the Wildlife Services, Wildlife Disease Program as well as the Attending Veterinarian for the National Wildlife Research Center (NWRC) in Fort Collins, Colorado. Prior to this position he served APHIS in various roles, including Senior Staff Veterinarian with the Chronic Wasting Disease Program; pathologist in the General Pathology and Pathology Investigations Section of the Pathobiology Laboratory at the National Veterinary Services Laboratories (NVSL) in Ames, Iowa; Port Veterinarian at the Port of Sweetgrass, Montana; and field Veterinary Medical Officer in Lawrenceburg, Kentucky and Logan, Utah. Gidlewski's research in domestic animal and wildlife diseases primarily includes avian influenza, brucellosis, foot and mouth disease, rabbit hemorrhagic disease, heartwater, tuberculosis, and chronic wasting disease. Gidlewski received an M.S. in poultry nutrition from Virginia Polytechnic Institute and State University, an M.S. in veterinary pathology from Iowa State University and a DVM from the University of Pennsylvania.

Nicholas Haley is an associate professor in the Department of Microbiology and Immunology at Midwestern University. His research has focused on chronic wasting disease (CWD) management in captive cervid populations through both live animal testing and selective breeding of animals thought to

be less susceptible to the disease. He has published extensively on topics related to CWD transmission, pathogenesis, testing, and management in cervids. He has been given "Friend of the Industry" awards from both the North American Elk Breeders and Whitetails of Wisconsin, and in 2021 received the Zoetis Distinguished Teacher award for outstanding teaching and achievement in the field of Veterinary Medicine. Haley received a DVM from Cornell University and a Ph.D. from Colorado State University.

Debbie McKenzie is an emeritus professor in the Department of Biological Sciences at the University of Alberta, where she also served as the Associate Dean of the Graduate Faculty of Science. Her research team used several different model systems, ranging from cell-free to cell culture to primary neuronal cultures to transgenic mice to deer, to address questions regarding the etiology and pathogenesis of chronic wasting disease (CWD) and the research is performed in the state-of-the art Centre for Prions and Protein Folding Diseases. Her research focuses on the role of Prnp genetics in susceptibility to prion infection, CWD strain characterization and evolution, intra- and inter-species transmission of CWD prions, and development of biomarkers for prion diseases. McKenzie received a Ph.D. in medical biochemistry from the University of Calgary and has been involved in prion disease research since 1988.

Rodrigo Morales is a Professor in the Department of Neurology at The University of Texas Health Sciences Center at Houston. He has 20 years of experience in the field of protein misfolding diseases, specifically in prion and Alzheimer's diseases. His main research topics involve the prion-like nature of Aβ aggregates in Alzheimer's disease, the contribution of peripheral tissues and blood in amyloid pathology, the study of environmental components contributing to chronic wasting disease (CWD) dissemination, the strain and species barrier phenomena in prion diseases, and the pathological interaction between amyloidogenic proteins. Morales received a Ph.D. from the University of Chile.

Michael W. Miller served as a wildlife veterinarian with the Colorado Division of Parks and Wildlife from 1989 until his retirement from State service in 2022. Miller has studied a variety of topics related to the ecology and management of infectious diseases affecting wildlife in Colorado and elsewhere. As a veterinarian and scientist, he has extensive experience trying to understand and control chronic wasting disease (CWD) in both captive and free-ranging cervids. Miller has received regional and international awards recognizing his achievements in advancing wildlife health sciences and management, including his work on CWD. Miller received a B.S. in zoology with a biochemistry minor, a DVM, and a PhD from Colorado State University. Miller's former employer, the Colorado Division of Wildlife has a long history of setting policies, regulations, plans, and management actions related to the control of CWD. He served on ad hoc working groups providing scientific opinions on CWD to the European Food Safety Authority on behalf of European Commission, and chaired the Western Association of Fish & Wildlife Agencies' Wildlife Heath Committee, which advised the group's directors on topics related to CWD and its control. He has given many scientific presentations and published numerous papers on CWD throughout his career.

Margo Pybus leads Alberta, Canada's provincial wildlife disease program area and directly or indirectly contributes to ongoing disease and management-related programs, policies, research, recommendations, and education/outreach. She initiated and leads Alberta's ongoing wildlife chronic wasting disease (CWD) program since it began in 1998, and contributes to a wide range of CWD provincial, national, and international research initiatives. A long-standing member of the Wildlife Disease Association and The Wildlife Society, Pybus recently received the Wildlife Disease Association Tom Thorne & Beth Williams Memorial Award for life-time achievement. She also is a former President of the Alberta Chapter of The Wildlife Society and recipient of the highest Chapter honors. Subsequent to her groundbreaking masters thesis work, a nematode species now bears her name. Pybus received a B.Sc. and M.Sc. from the University of Guelph and a Ph.D. in wildlife parasites and diseases from the University of Alberta.

Tiffany Wolf is an assistant professor of Ecosystem Health at the College of Veterinary Medicine and co-director of the Minnesota Center for Prion Research and Outreach (MNPRO) at the University of Minnesota (UMN). Prior to becoming faculty, she was an associate veterinarian of the Minnesota Zoo for 10 years. Wolf is a wildlife epidemiologist and veterinarian who works closely with natural resource managers and community partners to understand wildlife disease patterns with the goal of developing science-based strategies that mitigate their impacts on both wildlife populations and the people that depend on them. In much of her research, she partners with Tribal Nations and other Indigenous communities in addressing their research priorities on issues such as zoonotic disease emergence, dog health, and chronic wasting disease (CWD). Wolf is the recipient of the UMN Community Engaged Scholar Award, Academy of Excellence in Team Science Award, and McKnight Land Grant Professorship. Notably, the Team Science award was based on the accomplishments of the MNPRO team in research and education on CWD and other prion diseases. Wolf received a DVM from Louisiana State University and a PhD from UMN. As co-director of MNPRO, Wolf has provided public testimony to Minnesota legislators on CWD science and MNPRO research and outreach activities to inform management decisions related to the control of CWD in the state.

Appendix B
Public Meeting Agendas

COMMITTEE ON THE REVIEW OF TRANSMISSION AND GEOGRAPHIC SPREAD OF CHRONIC WASTING DISEASE IN U.S. CERVID POPULATIONS

The Keck Center, 500 Fifth Street, NW
Washington, DC 20001
October 9, 2023
ROOM 103

SESSION 1—OPEN

9:30	**Welcome** *Lonnie King, Committee Chair* Introductions, Statement of Objectives
9:45	**Discussion with Sponsors** A pre-recorded talk from the sponsors will be presented followed by Q&A *Camille Hopkins, USGS* *Dianne Sutton, USDA/APHIS* 1. Why did you ask for the study? 2. Who is the audience for the report? 3. What kinds of conclusions will be most helpful for your agency? 4. How do you plan to use the final report?
11:00	**Overview of the Scope and Scale of the CWD Problem** *Jason Bartz, Creighton University and Dan Walsh, U.S. Geological Survey* Dosage Testing CWD in the Environment
12:00	Working Lunch
1:00	**Socio-Economic impacts of CWD** *David P. Anderson, Texas A&M University* *Danielle Ufer, U.S. Department of Agriculture*
2:00	Break
2:15	**Group discussion Round Robin with Guests**: How well are wild and captive cervid management practices constrained by science? How well do we understand risk factors?
3:25	**Concluding Remarks-Chair**

COMMITTEE ON THE REVIEW OF TRANSMISSION AND GEOGRAPHIC SPREAD OF CHRONIC WASTING DISEASE IN U.S. CERVID POPULATIONS

The Keck Center, 500 Fifth Street, NW
Washington, DC 20001
November 16th, 2023
ROOM 105

SESSION 1—OPEN

9:30 **Welcome, Introductions, Statement of Objectives**
Lonnie King, Committee Chair

9:45 **Panel Discussion: How is CWD manifesting itself and affecting cervid farming?**
Shawn Schafer, Executive Director, North American Deer Farmer's Association
Sam Burgeson, Wildlife Research Center
Glen Zebarth, DVM and Elk Farmer

Each panelist will have 7-10 minutes to provide comments regarding issues they believe the committee should deliberate. The following prompting questions were provided as suggestions for their comments:

1. By what mechanisms do you believe CWD is introduced into captive populations and why do you believe this?
2. What management practices, voluntary or required, do you think are effective against the spread of CWD among management populations and why? Which do you think are ineffective and why?
3. What is the effect of CWD and CWD maintenance practices on herds?
4. What do you see is the future role of live and postmortem testing for CWD?
5. What keeps you up and night about CWD that you would like the committee to discuss?

11:00 **Break**

11:15 **Movement of CWD in Captive Herds: What does Surveillance Data Tell Us?**
Scott Wells, University of Minnesota

12:15 Working Lunch

1:00 **Conversation with Tracy A. Nichols**
Tracy A Nichols, Veterinary Services Cervid Health Program, United States Department of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, Colorado

Prompting questions

1. Please provide a brief description of the captive cervid program and how it is decided which management practices to implement.
2. What kinds of surveillance is conducted to determine the effectiveness of maintenance activities?
3. How and when is it decided that maintenance activities need to be modified? What is known but not published on the epidemiology of CWD in captive herds? What kinds of historical surveillance data are available and how does one get access to them?

2:00 **General Discussion: Broader Discussion on Future Directions for Control of CWD in Captive Herds**

2:30 **Discussion: Examples of How Cervids are Valued by Native Americans**
Tolani Francisco, DVM, USDA Forest Service

3:30 **Concluding Remarks—*Chair***

COMMITTEE ON THE REVIEW OF TRANSMISSION AND GEOGRAPHIC SPREAD OF CHRONIC WASTING DISEASE IN U.S. CERVID POPULATIONS

The Keck Center, 500 Fifth Street, NW
Washington, DC 20001
December 14th, 2023
ROOM 105

SESSION 1—OPEN

9:30 **Welcome**
Lonnie King, Committee Chair
Introductions, Statement of Objectives

9:45 **Presentation: Genomics and Biosecurity**
Christopher M. Seabury, Ph.D., Professor, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University

10:45 **Break**

11:00 **Panel Discussion 1: State Management of Free-Ranging Cervids**
Krysten Schuler, MS, Ph.D, Assistant Research Professor, Cornell University, Department of Public & Ecosystem Health, Cornell University College of Veterinary Medicine
Michelle Carstensen, Ph.D., Wildlife Health Program Supervisor, Wildlife Division, Minnesota Department of Natural Resources

12:15 Working Lunch

1:00 **Panel Discussion 2: State Management of Captive Cervids**
Jennifer R. Ballard, DVM, PhD, CWB, Arkansas Game and Fish Commission, State Wildlife Veterinarian/Assistant Chief (Research Division)
Hunter Reed, DVM, MPH, Wildlife Veterinarian, Texas Parks and Wildlife Department

2:15 **General Discussion: Broader discussion on future directions for control of CWD in States**

3:15-3:30 **Concluding remarks—*Chair***

Appendix C
Commonly Asked Questions[1]

What is chronic wasting disease?

Chronic wasting disease (CWD) is a contagious and fatal prion disease that damages the central nervous systems of cervids (e.g., species in the "deer family"), including free-ranging and captive white-tailed deer, mule deer, elk (also known as wapiti), reindeer, sika deer, red deer, and moose. Like all other prion diseases identified to date, CWD is a progressive transmissible spongiform encephalopathy—a type of disease characterized in its final stage by the microscopic, spongy-appearing degeneration in the brain tissue of affected animals (Williams and Young, 1980, 1992; Williams, 2005; Tranulis et al., 2021).

Where is CWD found?

As of August 1, 2024, CWD has been found in free-ranging and captive cervid populations in 35 U.S. states, 5 Canadian provinces, South Korea, Finland, Norway, and Sweden.

What causes CWD?

As with other transmissible spongiform encephalopathies, CWD in susceptible cervids results when the normal prion proteins, found in highest concentrations in the central nervous system, encounter misfolded variants of that protein. The misfolded protein causes the normal protein to misfold and become infectious itself. These misfolded prions are relatively resistant to degradation and thus accumulate in the infected animal's lymphatic and nervous systems, spreading to other tissues and organs via the lymphatic, nervous, and circulatory systems. The spread and accumulation of these misfolded prions damages the infected animal's brain and other organs, leading to behavioral abnormalities, loss of regular body functions, physical degradation, and ultimately death.

Where did CWD come from?

CWD's cause and emergence in North America cannot be traced with any reliability, but a clinical syndrome like CWD was observed in Colorado in 1967 and recognized as a spongiform encephalopathy in 1978. There are three hypotheses for how CWD originated: (1) there may have been a spontaneous misfolding of normal prions which led to the disease and transmission to other cervids; (2) CWD could have adapted to cervids exposed to scrapie—a prion disease found in sheep; and (3) CWD developed from exposure to an unidentified prion disease in another free-ranging or captive mammal and adapted to cervids. Scientists may never be able to determine which or what combinations of these is the source of CWD.

What species are susceptible to CWD?

CWD occurs naturally in captive and free-ranging members of the deer family (Cervidae). These include mule deer, black-tailed deer, white-tailed deer, North American elk, red deer, reindeer, and moose. CWD has been documented in various captive and experimentally inoculated cervids, including the species mentioned above, plus muntjac, Sika deer, and sika/red deer hybrids.

[1] This summary does not include references. Citations for the information presented herein are provided in the main body of the report (References).

What are the signs that an animal has CWD?

Clinical signs of CWD can differ within and between species, but for most of the long CWD disease course (typically 1.5-2.5 years in white-tailed deer, mule deer and reindeer; up to 4 years in elk), infected animals appear and behave normally. Infected animals in the later stages of CWD commonly exhibit progressive deterioration of body condition, repetitive movement, periods of somnolence (i.e., drowsiness), fixed stares, altered postures with lowered head and ears, and excess salivation with drooling.

The diagnosis of CWD cannot be made based on these clinical signs because other diseases and conditions exhibit similar signs. Instead, diagnosis relies on detecting evidence of CWD prions—through laboratory testing—in the brain or lymphoid tissues of animals.

How is CWD transmitted?

Routes of transmission and infection of CWD have not been determined definitively, but current research indicates that the disease is spread primarily through oral or oral-nasal routes via animal-to-animal contact. Susceptible animals can also be exposed to infectious prions though animal-to-animal interactions, or through contaminated environments (e.g., soil, water) that can remain a source of CWD prion exposure for many years. CWD prions are most likely shed via the saliva, feces, and urine of infected cervids, are found in decomposing carcasses of infected cervids, and in placental tissues collected from infected cervids.

How does CWD spread to new areas?

CWD's geographical expansion has been promoted by both human-caused and natural factors. Natural cervid movements and migration, social interactions, mating, and many other cervid behaviors (e.g., grooming, maternal interactions, congregation around food and water sources) may have significantly influenced current expansion of the form of CWD found in the United States to new free-ranging cervid populations. Human facilitated movement of infected animals or carcasses has introduced CWD to new regions and countries. The transportation of CWD-infected cervid parts by hunters, commercial sale of goods containing cervid products contaminated with CWD prions, and the transportation of hay and animal feed contaminated by infectious CWD prions from soil, feces, and cervid parts may contribute to CWD spread and prevalence.

Is it true that CWD prions can remain infectious outside the body in nature for a long time?

Due to their extreme resistance to degradation, CWD prions can persist and remain infectious in the environment for many years after being shed by a cervid (e.g., through defecation or urination). Some scientists speculate that the prions can remain infectious in some environments for decades where cervids can become exposed.

Can livestock or domestic cervids contract CWD?

Given that sheep and cattle are both susceptible to non-CWD prion diseases (scrapie in sheep and bovine spongiform encephalopathy in cattle), there has been concern that CWD could spillover to agriculturally important species. There is no evidence to date to suggest that sheep, cattle, pigs, or other domestic cervids can contract CWD through natural mechanisms. Various non-cervid species have been experimentally infected with CWD in the laboratory, but data so far suggest that there is a robust barrier between non-cervid species and known strains of CWD infection via natural transmission routes.

Can humans get CWD?

Multiple studies have failed to demonstrate evidence of humans contracting CWD or of links between CWD exposure and human prion disease. However, given the spread of CWD in cervid populations the potential for future spillover of CWD to humans cannot be discounted.

What is being done to control CWD and who is responsible?

In the United States, the responsibility of managing CWD in free-ranging cervid populations lies primarily with wildlife management agencies. These agencies commit millions of dollars annually to control and manage CWD by employing a host of strategies. To prevent transmission or introduction of CWD, agencies implement regulatory interventions such as live cervid and cervid carcass part movement restrictions, bans on baiting and feeding wild cervids, cervid part disposal restrictions, and mandatory submission of hunter harvested cervids for CWD surveillance programs. To detect and monitor the prevalence of CWD, agencies often require mandatory submission of hunter harvested cervids for disease testing and conduct weighted, risk-based surveillance strategies to detect early disease occurrences or outbreaks. If CWD is found in free-ranging cervids, the response actions of wildlife agencies include increased risk-based sampling to delineate disease-affected area, communication and engagement with interested and affected parties, initiation or expansion of CWD-related regulations, and initiation of CWD control measures such as modifications to hunter harvest rates, targeted culling, and mandatory submission of hunter harvested cervids.

Management of CWD in captive cervid populations primarily falls under the jurisdiction agricultural agencies that prioritize measures targeting the prevention of disease transmission. These include reliable means of both herd and animal identification, current records of animal inventory and movement, biosecurity protocols, accurate disease and surveillance. If CWD is discovered in a captive herd, management relies on herd-level quarantine, animal movement tracing, and in most cases depopulation with further work done to characterize the extent of the outbreak and minimize its impacts on surrounding wild cervid herds. Indemnity is provided where available to both compensate the herd owner and encourage disease reporting and subsequent control measures.

Why hasn't CWD been eradicated?

The disease agent of CWD is a misfolded protein; a non-living structure called a prion that is constructed of 256 amino acids. They contain no genetic material and cannot be neutralized with conventional pharmaceuticals or sanitizing techniques that are effective against bacterial, viral, fungal, and parasitic diseases. Infectious CWD prions are highly resistant to heat, chemicals, and enzymes, and can persist and remain infectious in the environment for many years. Because CWD prions are non-living proteins, no vaccines or therapeutics have yet been developed that can neutralize them in an infected animal. This, in combination with the disease's potentially long incubation period, results in an infected animal shedding infectious prions into the environment and directly to other cervids for months—and even years--before the cervid inevitably succumbs to the disease.

Appendix D
Published Diagnostic Testing Platforms

This appendix serves a resource to summarize information on published CWD diagnostic testing platforms discussed in Chapter 4. The table describes the testing platform's species and biological sample application, sensitivity, specificity, advantages and limitations.

TABLE D-1 Summary of Published Diagnostic Testing Platforms, Comparing Diagnostic Sensitivity, Specificity, Advantages and Limitations

Diagnostic Test	Tissue	Species	Study Design	Se	Sp	Advantages	Limitations	References
IHC	Obex	MD, WTD, Elk	O, E	77-100%	100%	Highly specific; High Se in later stages of disease due to delayed central nervous system involvement	Lower Se as compared to peripheral lymphoid tissues, or to amplification assays; RPLN likely more sensitive than obex in WTD and MD	Spraker et al., 2002 Keane et al. 2008 Haley et al.2009 Wyckoff et al. 2015 Miller and Williams, 2002
	RPLN	MD, WTD, Elk	O, E	88-99%	96-100%	Highly specific; RPLN likely more sensitive than obex in WTD and MD, especially in early disease stages; Biopsy can be used in official antemortem testing of WTD	Lower Se as compared to amplification assays	Miller and Williams, 2002 Keane et al. 2008 Haley et al. 2009 Picasso-Risso et al. 2022 Wyckoff et al. 2015
	RAMALT	WTD	O	68-80%	>99%	Highly specific	3-month lag in detection as compared to RT-QuIC; Sample quality impacted by number of follicles obtained in biopsy; lower detection odds in wt/G96S deer as compared to wt/wt deer	Keane et al. 2009 Thomsen et al. 2012 Henderson et al. 2020
	Tonsil	MD, WTD	O	93-99%	100%	Highly specific; Biopsy can be used in official antemortem testing of WTD	3-month lag in detection as compared to RT-QuIC	Spraker et al. 2002 Miller and Williams, 2002 Keane et al. 2009 Henderson et al. 2020
ELISA	Obex	MD, WTD, Elk	O	92-93%	100%	Rapid test; High level of agreement with IHC	Disagreement with IHC tends to be in early stages of disease	Hibler et al. 2003
	RPLN	MD, WTD, Elk	O	98-100%	>99%	Rapid test; High level of agreement with IHC	Disagreement with IHC tends to be in early stages of disease	Hibler et al. 2003

continued

TABLE D-1 *continued*

Diagnostic Test	Tissue	Species	Study Design	Se	Sp	Advantages	Limitations	References
PMCA	Obex	Elk	O	95%	94%	Higher Se as compared to IHC of obex and RPLN	Cross-contamination during sample processing can reduce Sp (down to 62%)	Wyckoff et al. 2015
	Tonsil	WTD	E	NA	NA	Higher Se in earlier stages of disease as compared to IHC	Se and Sp never formally estimated	Haley et al. 2012
	Blood	WTD	O	79.3%	100%	100% specificity with clinical disease	Low sensitivity (53%) in nonclinical, early stage (*i.e.*, IHC+ lymph node only) disease	Kramm et al. 2017
	Feces	WTD	O	55-100%	98%	Highly sensitive for animals at late stages of pre-clinical disease and wt genotype	Sensitivity decreases depending on the stage of the incubation period and polymorphic variations in the *PRNP* gene.	Bravo-Risi et al. 2023
RT-QuIC	RPLN	MD, WTD, Moose	O	100%	100%	May be more sensitive than IHC or ELISA at earlier stages of disease; Under evaluation for official test validation	Limited number of positive samples (n=23, all MD or WTD) and comparison restricted to one tissue. Requires more validation studies to fully estimate Se and Sp in absence of a "gold standard" comparison	Haley et al. 2014
	Tonsil	WTD	O	89-95%	96-98%	Accessible for antemortem testing	Earlier detection as compared to IHC	Picasso-Risso et al. 2022 Henderson et al. 2020
	RAMALT	WTD, Elk	O	70-92%	94-100%	Under evaluation for official test validation for antemortem testing	Lower Se in earlier stages of disease; Earlier detection as compared to IHC	Haley et al. 2016a Haley et al. 2016b Manne et al. 2017 Henderson et al. 2020 Piel et al. 2024

	Ear pinna	MD, WTD	O	81-95%	91-100%	Easily accessible for antemortem testing	Mixed findings in regard to whether biopsy location on pinna affects Se	Ferreira et al. 2021; Burgener et al. 2022
	Nasal secretions/ brushings	WTD, Elk	O, E	16-56%	90-100%	NAPTA-precipitation enhanced Se (up to 56%)	Seeding activity and detection follows tonsil biopsy seeding activity; Highest sensitivity in terminal stages of disease	Kraft et al. 2023, Haley et al. 2016a; Haley et al. 2016b
	Third eyelid	WTD, Elk	O, E	72-96%	100%	Higher Se as compared to IHC of same tissue	Lower Se observed in naturally infected elk; Low numbers of animals tested	Cooper et al. 2019
MN-QuIC	RPLN	WTD	O	96%	100%	Field-deployable testing equipment; Rapid turnaround time to test result	Low numbers of animals tested	Christenson et al. 2022

NOTE: Direct comparisons of sensitivity and specificity estimates reported across publications are complicated by differences in sample sources and numbers, study design, and potentially other variables. As a general pattern, results from different testing platforms and main tissue sampling sites tend to converge as disease progresses in infected individuals.

Se: Sensitivity, defined as the probability of a CWD-positive cervid testing positive; Sp: Specificity, defined as the probability of a CWD-negative cervid testing negative; RPLN: retropharyngeal lymph nodes; RAMALT: rectoanal mucosa-associated lymph tissue; WTD: white-tailed deer; MD: mule deer; O: Observational study with samples collected from naturally infected cervids; E: experimental study with samples collected from experimentally infected cervids.

REFERENCES

Bravo-Risi, F., P. Soto, R. Benavente, T.A. Nichols, and R. Morales. 2023. Dynamics of CWD Prion Detection in Feces and Blood from Naturally Infected White-Tailed Deer. *Scientific Reports* 13:20170. https://doi.org/10.1038/s41598-023-46929-9.

Burgener, K.R., S.S. Lichtenberg, A. Lomax, D. J. Storm, D.P. Walsh, and J.A. Pedersen. 2022. Diagnostic testing of chronic wasting disease in white-tailed deer (Odocoileus virginianus) by RT-QuIC using multiple tissues. *PLOS ONE* 17(11):e0274531. https://doi.org/10.1371/journal.pone.0274531.

Christenson, P.R., M. Li, G. Rowden, M.D. Schwabenlander, T.M. Wolf, S.H. Oh, and P.A. Larsen. 2022. A field-deployable diagnostic assay for the visual detection of misfolded prions. *Scientific Reports* 12(1):12246. https://doi.org/10.1038/s41598-022-16323-y.

Cooper, S.K., C.E. Hoover, D.M. Henderson, N.J. Haley, C.K. Mathiason, and E.A. Hoover. 2019. Detection of CWD in cervids by RT-QuIC assay of third eyelids. *PLOS ONE* 14(8):e0221654. https://doi.org/10.1371/journal.pone.0221654.

Ferreira, N.C., J.M. Charco, J. Plagenz, C.D. Orru, N.D. Denkers, M.A. Metrick, A.G. Hughson, K.A. Griffin, B. Race, E.A. Hoover, J. Castilla, T.A. Nichols, M.W. Miller, and B. Caughey. 2021. Detection of chronic wasting disease in mule and white-tailed deer by RT-QuIC analysis of outer ear. *Scientific Reports* 11(1):7702. https://doi.org/10.1038/s41598-021-87295-8.

Haley, N.J., C. Siepker, L.L. Hoon-Hanks, G. Mitchell, W.D. Walter, M. Manca, R.J. Monello, J.G. Powers, M.A. Wild, E.A. Hoover, B. Caughey, and J.A. Richt. 2016b. Seeded Amplification of Chronic Wasting Disease Prions in Nasal Brushings and Recto-anal Mucosa-Associated Lymphoid Tissues from Elk by Real-Time Quaking-Induced Conversion. *The Journal of Clinical Microbiology* 54(4):1117-26. https://doi.org/10.1128/jcm.02700-15.

Haley, N.J., C. Siepker, W.D. Walter, B.V. Thomsen, J.J. Greenlee, A.D. Lehmkuhl, and J.A. Richt. 2016a. Antemortem Detection of Chronic Wasting Disease Prions in Nasal Brush Collections and Rectal Biopsy Specimens from White-Tailed Deer by Real-Time Quaking-Induced Conversion. *The Journal of Clinical Microbiology* 54(4):1108-16. https://doi.org/10.1128/jcm.02699-15.

Haley, N.J., C.K. Mathiason, M.D. Zabel, G.C. Telling, and E.A. Hoover. 2009. Detection of Sub-Clinical CWD Infection in Conventional Test-Negative Deer Long after Oral Exposure to Urine and Feces from CWD+ Deer. *PLOS ONE* 4(11):e7990. https://doi.org/10.1371/journal.pone.0007990.

Haley, N.J., C.K. Mathiason, S. Carver, G.C. Telling, M.D. Zabel, and E.A. Hoover. 2012. Sensitivity of protein misfolding cyclic amplification versus immunohistochemistry in ante-mortem detection of chronic wasting disease. *Journal of General Virology* 93(Pt 5):1141-1150. https://doi.org/10.1099/vir.0.039073-0.

Haley, N.J., S. Carver, L.L. Hoon-Hanks, D.M. Henderson, K.A. Davenport, E. Bunting, S. Gray, B. Trindle, J. Galeota, I. LeVan, T. Dubovos, P. Shelton, and E.A. Hoover. 2014. Detection of chronic wasting disease in the lymph nodes of free-ranging cervids by real-time quaking-induced conversion. *Journal of Clinical Microbiology* 52(9):3237-43. https://doi.org/10.1128/jcm.01258-14.

Henderson, D.M., N.D. Denkers, C.E. Hoover, E.E. McNulty, S.K. Cooper, L.A. Bracchi, C.K. Mathiason, and E.A. Hoover. 2020. Progression of chronic wasting disease in white-tailed deer analyzed by serial biopsy RT-QuIC and immunohistochemistry. *PLOS ONE* 15(2):e0228327. https://doi.org/10.1371/journal.pone.0228327.

Hibler, C.P., K.L. Wilson, T.R. Spraker, M.W. Miller, R.R. Zink, L.L. DeBuse, E. Andersen, D. Schweitzer, J.A. Kennedy, L.A. Baeten, J.F. Smeltzer, M.D. Salman, and B.E. Powers. 2003. Field validation and assessment of an enzyme-linked immunosorbent assay for detecting chronic wasting disease in mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus), and Rocky Mountain elk (Cervus elaphus nelsoni). *Journal of Veterinary Diagnostic Investigation* 15(4):311-9. https://doi.org/10.1177/104063870301500402.

Keane, D., D. Barr, R. Osborn, J. Langenberg, K. O'Rourke, D. Schneider, and P. Bochsler. 2009. Validation of use of rectoanal mucosa-associated lymphoid tissue for immunohistochemical diagnosis of chronic wasting disease in white-tailed deer (Odocoileus virginianus). *Journal of Clinical Microbiology* 47(5):1412-7. https://doi.org/10.1128/jcm.02209-08.

Keane, D.P., D.J. Barr, J.E. Keller, S.M. Hall, J.A. Langenberg, and P.N. Bochsler. 2008. Comparison of retropharyngeal lymph node and obex region of the brainstem in detection of chronic wasting disease in white-tailed deer (Odocoileus virginianus). *Journal of Veterinary Diagnostic Investigation*. 20(1):58-60. https://doi.org/10.1177/104063870802000110.

Kraft, C.N., N.D. Denkers, C.K. Mathiason, and E.A. Hoover. 2023. Longitudinal detection of prion shedding in nasal secretions of CWD-infected white-tailed deer. *Journal of General Virology* 104(1). https://doi.org/10.1099/jgv.0.001825.

Kramm, C., S. Pritzkow, A. Lyon, T. Nichols, R. Morales, and C. Soto. 2017. Detection of Prions in Blood of Cervids at the Asymptomatic Stage of Chronic Wasting Disease. *Scientific Reports*. 7 (1): 17241. https://doi.org/10.1038/s41598-017-17090-x.

Manne, S., N. Kondru, T. Nichols, A. Lehmkuhl, B. Thomsen, R. Main, P. Halbur, S. Dutta, and A.G. Kanthasamy. 2017. Ante-mortem detection of chronic wasting disease in recto-anal mucosa-associated lymphoid tissues from elk (Cervus elaphus nelsoni) using real-time quaking-induced conversion (RT-QuIC) assay: A blinded collaborative study. *Prion* 11(6):415-430. https://doi.org/10.1080/19336896.2017.1368936.

Picasso-Risso, C., M.D. Schwabenlander, G. Rowden, M. Carstensen, J.C. Bartz, P.A. Larsen, and T.M. Wolf. 2022. Assessment of Real-Time Quaking-Induced Conversion (RT-QuIC) Assay, Immunohistochemistry and ELISA for Detection of Chronic Wasting Disease under Field Conditions in White-Tailed Deer: A Bayesian Approach. *Pathogens* 11(5). https://doi.org/10.3390/pathogens 11050489.

Piel, R.B. 3rd, S.E. Veneziano, E.M. Nicholson, D.P. Walsh, A.D. Lomax, T.A. Nichols, C.M. Seabury, and D.A. Schneider. 2024. Validation of a real-time quaking-induced conversion (RT-QuIC) assay protocol to detect chronic wasting disease using rectal mucosa of naturally infected, pre-clinical white-tailed deer (Odocoileus virginianus). *PLOS ONE* 19(6):e0303037. https://doi.org/10.1371/journal.pone.0303037.

Spraker, T.R., K.I. O'Rourke, A. Balachandran, R.R. Zink, B.A. Cummings, M.W. Miller, and B.E. Powers. 2002b. Validation of monoclonal antibody F99/97.6.1 for immunohistochemical staining of brain and tonsil in mule deer (Odocoileus hemionus) with chronic wasting disease. *Journal of Veterinary Diagnostic Investigation* 14(1):3-7. https://doi.org/10.1177/104063870201400102.

Thomsen, B.V., D.A. Schneider, K.I. O'Rourke, T. Gidlewski, J. McLane, R.W. Allen, A.A. McIsaac, G.B. Mitchell, D.P. Keane, T.R. Spraker, and A. Balachandran. 2012. Diagnostic accuracy of rectal mucosa biopsy testing for chronic wasting disease within white-tailed deer (Odocoileus virginianus) herds in North America: effects of age, sex, polymorphism at PRNP codon 96, and disease progression. *Journal of Veterinary Diagnostic Investigation* 24(5):878-87. https://doi.org/10.1177/1040638712453582.

Wyckoff, A.C., N. Galloway, C. Meyerett-Reid, J. Powers, T. Spraker, R. J. Monello, B. Pulford, M. Wild, M. Antolin, K. VerCauteren, and M. Zabel. 2015. Prion amplification and hierarchical Bayesian modeling refine detection of prion infection. *Scientific Reports* 5:8358. https://doi.org/10.1038/srep08358.

Appendix E
Supplementary Information on Other TSEs and Their Economic Impacts

This appendix provides information about scrapie and bovine spongiform encephalopathy (BSE). It begins with a description their transmission and control and concludes with a discussion on their economic impacts.

SCRAPIE

Scrapie has affected sheep and goat herd for centuries, and transmission of the infectious prion through the movement of subclinical, infected animals between herds and the direct transmission and long-term environmental persistence within herds has complicated control. However, the identification of genetic-based resistance to infection has inspired control programs aimed at eradication of classical scrapie from sheep and goat herds (Goldman et al., 1994; Hunter et al., 1996; Hunter et al., 1997; Nodelijk et al., 2011; Spiropoulus et al., 2007). Existing control programs are based on the detection of classical scrapie in slaughtered or animals showing clinical signs of scrapie, followed by tracing and enhanced surveillance, selective culling, and replacement with genetically resistant stock. Success has been observed with these strategies, particularly in the United States, but country-wide eradication has yet to be realized (USDA Animal and Plant Health Inspection Service, 2023). Given concerns related to unintended consequences of genetic selection, as well as animal welfare and economic losses related to culling, there is growing interest in antemortem testing for early detection and selective removal. These existing and potential strategies are described in more detail here.

In the United States, a Cooperative State-Federal Scrapie Eradication Program was first enacted as an emergency measure in 1952 for the control of classical scrapie (United States Livestock Sanitary Association, 1959; Wineland, 1993), with the goal of declaring the U.S. goat herds and sheep flocks free of scrapie. Scrapie had been first reported in the United States in 1947, but the disclosure of additional infected flocks during 1952-1953 prompted the program's start up. By late 1961 scrapie had been reported in 105 flocks in 87 counties across 26 states (United States Livestock Sanitary Association, 1961). A decade later, cases had been diagnosed in 199 flocks from 30 states (United States Livestock Sanitary Association, 1971). A general trend of increase in the proportion of scrapie-infected flocks nationwide continued into the early 1990s (Wineland, 1993) and beyond.

Today, the eradication program is based on 1) education for increased scrapie awareness and prevention, 2) individual identification of sheep and goats and compliance with program standards, 3) scrapie surveillance through the Regulatory Scrapie Slaughter Surveillance program (RSSS), the Scrapie Free Flock Certification Program (SFCP), the Post Exposure Monitoring and Management Plan (PEMMP), and other on-farm, disease investigations (USDA National Scrapie Surveillance Plan, 2022) involving tracing, testing and clean-up, and 4) selective breeding for genetics-based scrapie resistance (USDA Animal and Plant Health Inspection Service, 2023). To achieve its goal of classical scrapie eradication, there would need to be no detection of classical scrapie through the established surveillance programs for a minimum of seven years. The success of the eradication program has been seen through the substantial reduction in scrapie incidence resulting from ongoing surveillance and swift action, including removal of infected animals and tracing based on animal identification, combined with the genetic selection and repopulation for herd resistance. The reduction in observed incidence since initiation of the RSSS program in 2003 suggests that the program may be close to meeting its eradication goal (USDA Animal and Plant Health Inspection Service, 2023). RSSS recently reported only the detection of

the atypical form of scrapie during 2022 surveillance, and the last detection of the infectious, classical form of scrapie targeted for control was made in 2021 among sheep and among goats in 2019 (USDA Animal and Plant Health Inspection Service, 2023) As of the 2022 report, 44 states have been free of classical scrapie in sheep and 48 free among goats for at least seven years. As incidence has declined, however, maintaining program momentum, ensuring compliance, and addressing the genetic diversity of the U.S. sheep and goat populations are ongoing concerns. Additionally, balancing disease control with animal welfare and economic considerations is a complex challenge.

The U.S. National Scrapie Eradication Program is expected to identify cases of atypical scrapie (Nor98-like scrapie). However, USDA-APHIS and the World Organization for Animal Health (WOAH) considers atypical scrapie to be "clinically, pathologically, biochemically, and epidemiologically unrelated to classical scrapie, may not be contagious and may, in fact, be a spontaneous degenerative condition of older sheep, (USDA National Scrapie Surveillance Plan. 2022.)" Therefore, USDA-APHIS does not respond to or control cases of atypical surveillance as is done for classical scrapie.

BOVINE SPONGIFORM ENCEPHALOPATHY

The first cases of classical bovine spongiform encephalopathy (BSE) in Great Britain were detected in 1986 and were quickly linked to the feeding of animal protein and bone meal to livestock (Wilesmith et al., 1988). The subsequent banning of animal protein in livestock feed was highly effective in reducing the classical BSE epidemic, with peak cases (37,280) occurring in 1992 and declining thereafter. Current World Organization for Animal Health (WOAH[1]) guidelines for the prevention and control of classical BSE[2] are based on risk of entry and exposure to classical BSE via cattle or product trade. Country or area risk is based on a risk assessment that considers the likelihood for BSE to be transmitted through the bovine population, ongoing surveillance for BSE within the country, and the ongoing occurrence and management of classical and atypical BSE cases (WOAH[1]). Atypical BSE[3] is not a WOAH listed disease, but members must destroy any atypical BSE cases and ensure that their carcasses do not enter feed or the food chain (WOAH[1]). The European Union also has stringent controls in place related to feed bans (i.e., banning animal protein in livestock feed), surveillance (e.g., testing emergency slaughter or fallen stock 24 or 48 months of age), and removal of specified risk materials (SRM) from human food chain (EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention Control), 2014). To test the efficacy of these control strategies in the protection of human health, a model of risk reduction as a function of cattle testing at slaughter and removal of SRM in Great Britain after the BSE outbreak suggested extremely low exposure levels to BSE contaminated material among the British human population (Adkin et al., 2010).

In the United States, BSE surveillance began in 1990[3], and only one case of classical BSE—associated with an import from Canada in 2003—has been detected.[4] Surveillance was enhanced from 2004-2006 (after the initial classical BSE detection) and later modified to an ongoing surveillance program focusing on cattle at higher risk of having BSE (USDA Animal and Plant Health Inspection Service, 2006). The United States relies on WOAH[1] import standards for BSE to prevent introduction through importation. Feeding mammalian protein to cattle has been banned since 1997. In 2008, BSE control regulations were enhanced to prevent the inclusion of SRM (i.e., brain and spinal cord, skull eyes, trigeminal nerve ganglia and dorsal root ganglia from animals 30 months or older, tonsils and distal ileum from all ages) in animal feed and prohibits carcass use if it has not been inspected and passed for human consumption, unless cattle are less than 30 months of age or SRM are removed.

[1] See https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_bse.htm (accessed May 31, 2024).
[2]. See https://www.aphis.usda.gov/nvap/reference-guide/control-eradication/bse (accessed August 28, 2024).
[3]See https://www.aphis.usda.gov/sites/default/files/fs-bse.pdf (accessed August 28, 2024).
[4] See https://www.aphis.usda.gov/livestock-poultry-disease/cattle/bse (accessed August 28, 2024).

ECONOMIC IMPACTS OF TSEs

The control of scrapie and BSE involves measures such as culling infected or susceptible sheep, goats or cattle, surveillance, and implementing strict feeding regulations to prevent the spread of the disease result in economic losses and costs associated with testing and surveillance to both producers and regulatory agencies. The USDA National Scrapie Surveillance plan (2022) describes the economic impacts of scrapie: "The presence of scrapie in the United States is estimated to cost American sheep producers $10 million to $20 million per year, principally in lost exports of sheep and goat products and breeding stock, semen, and embryos; decreased value of and, in some cases, increased expenditures for offal and carcass disposal; and increased production costs." However, the economic impacts of control are balanced by the benefits of consumer confidence in food safety and free trade of animal and animal products (Mattson et al., 2007). For example, trade-restrictions associated with BSE have caused substantial declines in the export of beef and live cattle, which lowers cattle and beef prices (Mattson et al., 2007). However, WOAH recognition as a controlled-risk country for either scrapie or BSE opens market access without trade restrictions for sheep and goats or cattle, respectively.

Following a 2003 BSE case in Washington state (CDC, 2004), losses from U.S. trade restrictions were estimated at $3.2-4.7 billion (Coffey et al., 2005). Coffey et al., (2005) offers a perspective on the various economic impacts of BSE in the US from loss of trade and associated BSE control policies. For example, voluntary testing was considered by some US producers to gain renewed access international markets, but such a strategy and its associated costs were recognized to differentially impact different sized cattle production companies (based on direct costs, market access, and risk of identifying a case with resulting market closures). Exclusion of SRM material from products for human consumption have also been recognized to impact profits, where exclusion of non-ambulatory cattle alone in 2004 was estimated to be $64.6M (Coffey et al., 2005). Similarly, the cost of banning SRM from animal feed was estimated at $2.16 per head (Coffey et al., 2005).

A cost and benefit analysis of a scrapie using an antemortem test and remove program (using two testing strategies: single year (ST) and multi-year (MT) testing) for sheep in Great Britain was conducted in comparison to the existing Compulsory Scrapie Flock Scheme (CSFS) program (removal of susceptible and replacement with resistant genotypes in scrapie affected herds) (Boden et al., 2012). The study demonstrated the cost effectiveness of single test strategy, but reenforced previous findings that the CSFS strategy was most effective for control. Among high-risk flocks, MT cost about the same as the CSFS program and was only slightly less effective if test sensitivity was at or above 90%. None of the programs were cost-effective in low or medium risk flocks where disease resistance was high. However, as flocks had increasingly higher proportions of genetic resistance, the ST approach became more cost-effective than the MT or CSFS programs.

Evaluations of the economic impacts associated with consumer confidence in product safety after the detection of BSE cases have also been conducted. Studies have found that case detection has led to lower consumer demand impacting markets by lowering the price of beef, particularly early on, but recovery has also been observed (Burton and Young 1996; Ding et al., 2011; Mu et al., 2015). However, following the BSE case in Canada in 2003, consumer responses varied in surprising ways (increased consumer interest at first, followed by a decrease) (Yang and Goddard, 2011). These variations in response to BSE were found to be associated with risk perception and media coverage. Collectively, these studies demonstrate that consumer response and market demand might be expected to change after the occurrence of cases of TSEs with known zoonotic risk, although the direction of that impact (positive vs negative) and the duration may be difficult to predict.

REFERENCES

Adkin, A., V. Webster, M.E. Arnold, G.A.H. Wells, and D. Matthews. 2010. Estimating the impact on the food chain of changing bovine spongiform encephalopathy (BSE) control measures: The BSE Control Model. *Preventive Veterinary Medicine* 93(2):170-182.

Boden, L., I. Handel, N. Hawkins, F. Houston, H. Fryer, and R. Kao. 2012. An Economic Evaluation of Preclinical Testing Strategies Compared to the Compulsory Scrapie Flock Scheme in the Control of Classical Scrapie. *PLOS ONE* 7(3):e32884, https://doi.org/10.1371/journal.pone.0032884.

Burton, M., and T. Young. 1996. The impact of BSE on the demand for beef and other meats in Great Britain. *Applied Economics* 28(6):687-693. https://doi.org/10.1080/000368496328434.

CDC. 2004. Bovine spongiform encephalopathy in a dairy cow–Washington State. *Morbidity and Mortality Weekly Report* 52(53):1280-5.

Coffey, B., J. Mintert, J. Fox, T. Schroeder, and L. Valentin. 2005. The economic impact of BSE on the US beef industry: Product value losses, regulatory costs, and consumer reactions. (Extension Bulletin MF-2678). Kansas State University Agricultural Experiment Station and Cooperative Extension Service. Manhattan, KS.

Ding, Y., M.M. Veeman, and W.L. Adamowicz. 2011. Habit, BSE, and the Dynamics of Beef Consumption. *Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie* 59(3):337-359. https://doi.org/10.1111/j.1744-7976.2010.01205.x.

Goldmann, W., N. Hunter, G. Smith, J. Foster, and J. Hope. 1994. PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *Journal of General Virology* 75(Pt 5):989-95. https://doi.org/10.1099/0022-1317-75-5-989.

Hunter, N. 1997. PrP genetics in sheep and the implications for scrapie and BSE. *Trends in Microbiology* 5(8):331-334. https://doi.org/https://doi.org/10.1016/S0966-842X(97)01081-0.

Hunter, N., J.D. Foster, W. Goldmann, M.J. Stear, J. Hope, and C. Bostock. 1996. Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. *Archives of Virology* 141(5):809-24. https://doi.org/10.1007/bf01718157.

Mattson, J.W. and W.W. Koo. 2007. Effects of Bovine Spongiform Encephalopathy Outbreaks on U.S. Cattle and Beef Prices. *Review of Agricultural Economics* 29(4):734-748.

Mu, J.E., B.A. McCarl, A. Hagerman, and D. Bessler. 2015. Impacts of bovine spongiform encephalopathy and avian influenza on U.S. meat demand. *Journal of Integrative Agriculture* 14(6):1130-1141. https://doi.org/10.1016/S2095-3119(14)60996-5.

Nodelijk, G., H.J. van Roermund, L.J. van Keulen, B. Engel, P. Vellema, and T.J. Hagenaars. 2011. Breeding with resistant rams leads to rapid control of classical scrapie in affected sheep flocks. *Veterinary Research* 42(1):5. https://doi.org/10.1186/1297-9716-42-5.

Spiropoulos, J., C. Casalone, M. Caramelli, and M.M. Simmons. 2007. Immunohistochemistry for PrPSc in natural scrapie reveals patterns which are associated with the PrP genotype. *Neuropathology and Applied Neurobiology* 33(4):398-409. https://doi.org/10.1111/j.1365-2990.2007.00800.x.

United States Livestock Sanitary Association. 1959. Sixty-Third Annual Meeting of the United States Livestock Sanitary Association. San Francisco, California.

United States Livestock Sanitary Association. 1961. Sixty-Fifth Annual Meeting of the United States Livestock Sanitary Association. Minneapolis, Minnesota.

United States Livestock Sanitary Association. 1971. Seventy-Fifth Annual Meeting of the United States Livestock Sanitary Association. Oklahoma City, Oklahoma.

USDA Animal and Plant Health Inspection Service. 2006. Bovine Spongiform Encephalopathy (BSE) Ongoing Surveillance Plan. https://www.aphis.usda.gov/sites/default/files/BSE_ongoing_surv_plan_final.pdf (accessed August 28, 2024).

USDA Animal and Plant Health Inspection Service. 2023. National Scrapie Eradication Program Fiscal Year 2022 Report: October 1, 2021, to September 30, 2022. Strategy and Policy Unit Animal and Plant Health Inspection Service-Veterinary Services, Sheep and Goat Health Center U.S. Department of Agriculture. https://www.aphis.usda.gov/sites/default/files/scrapie-annual-report.pdf.

USDA National Scrapie Surveillance Plan. 2022. Animal and Plant Health Inspection Service, United States Department of Agriculture.

Wilesmith, J.W., G.A. Wells, M.P. Cranwell, and J.B. Ryan. 1988. Bovine spongiform encephalopathy: epidemiological studies. *Veterinary Record* 123(25):638-44.

Wineland, N.E. 1993. Epidemiology of reported scrapie in the United States: 1947-1991. Master of Science, Clinical Sciences Department, Colorado State University.

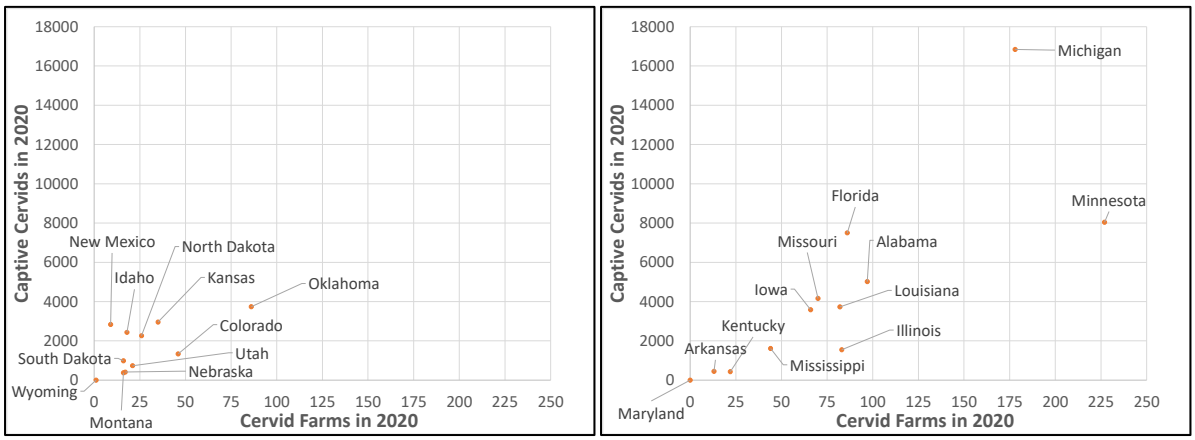
Yang, J., and E. Goddard. 2011. Canadian Consumer Responses to BSE with Heterogeneous Risk Perceptions and Risk Attitudes. *Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie* 59(4):493-518. https://doi.org/10.1111/j.1744-7976.2011.01225.x.

Appendix F
Data on Cervid Farms and Captive Cervids by State and Costs

This appendix provides an expanded visualization of the cervid data reported in Chiavacci (2022). It also provides descriptions on cervid numbers, number of paid hunting licenses, and years since first detection in wild cervid populations. The committee developed several sets of figures presenting relationships of these data, separated into two frames—states with mule deer and states without mule deer. The appendix concludes with a discussion of public agency costs.

DATA ON CERVID FARMS AND CAPTIVE CERVIDS

Chiavacci (2022) documents the number of cervid farms and captive cervids by state in 2020. Figure F-1 groups those in states with mule deer habitat (Figure F-1a) and those that do not (Figure F-1b). Most states have less than 150 cervid farms and less than 10,000 captive cervids. The number of cervid farms and captive cervids in Texas exceeds that of others states by over an order of magnitude. Most of the Great Plains and western states with mule deer have less than 50 farms and 3000 captive cervids. There are, on average, more cervid farms and captive cervids in states with only white-tailed deer habitat (i.e., the central and eastern states).

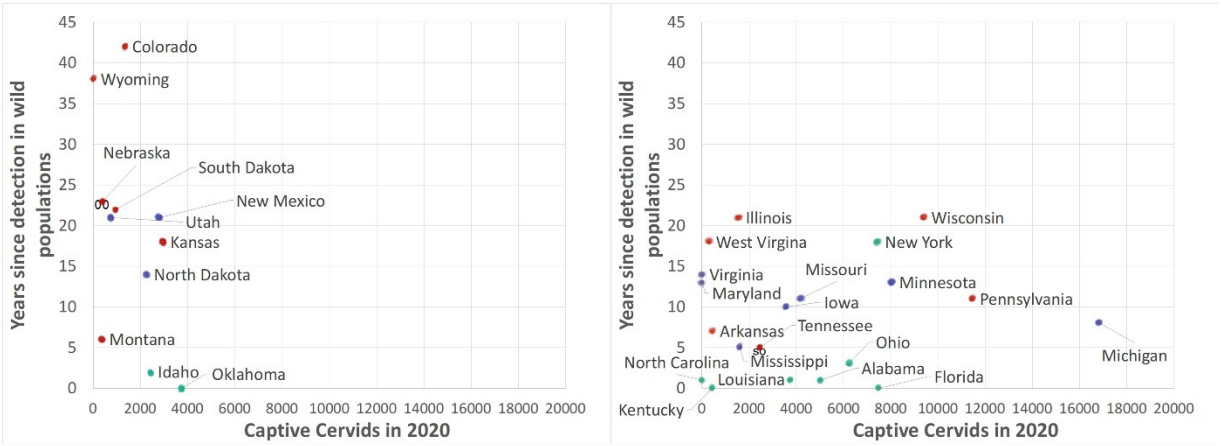


(a) States with mule deer* (b) States without mule deer

FIGURE F-1 Relationships between the number of cervid farms and number of captive cervids by state based on data in Chiavacci (2022; Table 1).*Texas had significantly more cervid farms and captive cervids than any other state with 1,498 cervid farms and 117,120 farmed cervids in 2020. Texas is excluded from panel (a) to keep the two panels comparable.

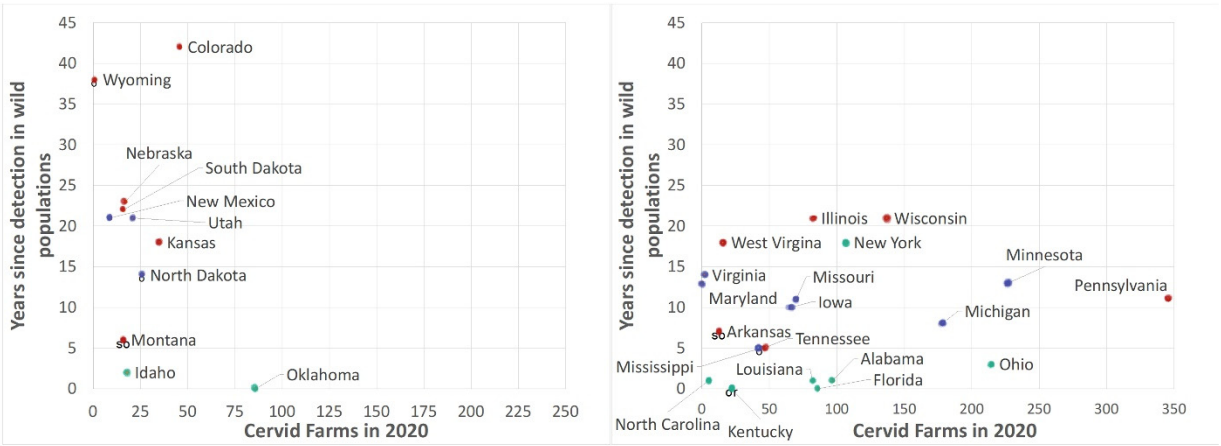
Some states such as Texas have both breeding and breeding and hunting operations. Little data exist on the scale of those operations apart from the facilities surveyed by Outlaw et al. (2017). Outlaw (2017) determined that the average breeding farm size was found to be 21 acres and 30 acres for breeding and hunting. The size of these facilities are much smaller than those in Alberta documented by Arnot et al. (2009), which are in the hundreds of acres.

Figure F-2 plots the years since first detection of CWD in wild cervids in each state against captive cervids in each state in 2020 in panels (Figure F-2a) and (Figure F-2b), and against the number of cervid farms in 2020, in panels (Figure F-2c) and (Figure F-2d).



(a) Captive cervids, states* with mule deer

(b) Captive cervids, states without mule deer



(c) Cervid farms, states* with mule deer

(d) Cervid farms, states without mule deer

FIGURE F-2 Years since first detection in wild populations, number of cervid farms and number of captive cervids by state in 2020. Data on years since detection in wild cervid populations were obtained from state wildlife agency and related websites. [a] The color of dots for each state reflects the level of endemic CWD as assigned by the committee: red dots indicate states with endemic CWD in 20 or more locales; blue dots indicate states with CWD that is endemic in fewer than twenty locales; green dots indicate states with a few cases. Texas (where CWD was first detected in wild populations 11 years ago, and which currently has endemic CWD in 20 or more locales) is excluded from panels (a, c) to keep the panels comparable. Data on cervid farms and captive cervids in each state in 2020 are from Chiavacci, 2022.

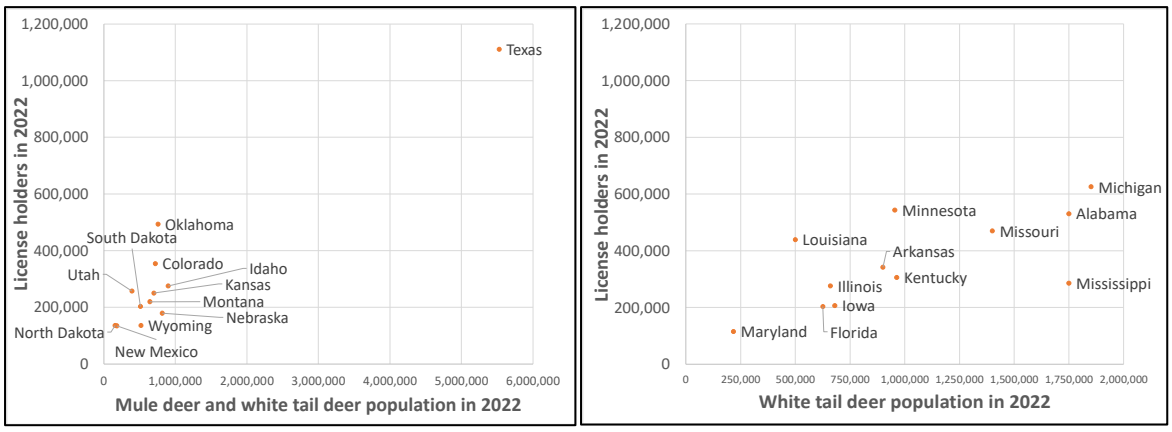
[a] Information on year of CWD detection in States:
Alabama, see https://www.outdooralabama.com/cwd/cwd-alabama.
Arkansas, see https://www.agfc.com/hunting/deer/chronic-wasting-disease/cwd-in-arkansas/.
California, see https://wildlife.ca.gov/.
Colorado, see https://www.cdc.gov/prions/cwd/occurrence.html.
Florida, see https://www.cidrap.umn.edu/chronic-wasting-disease/cwd-detected-first-time-florida-which-becomes-31st-affected-state.
Idaho, see https://idfg.idaho.gov/press/hunters-asked-help-chronic-wasting-disease-testing-salmon-region.
Illinois, see https://dnr.illinois.gov/content/dam/soi/en/web/dnr/programs/cwd/documents/cwd-fact-vs-fiction.pdf.

Indiana, see https://www.in.gov/dnr/fish-and-wildlife/wildlife-resources/wildlife-diseases-in-indiana/chronic-wasting-disease-cwd/.
Iowa, see https://www.iowadnr.gov/Hunting/Deer-Hunting/Deer-Health/Chronic-Wasting-Disease.
Kansas, see https://ksoutdoors.com/Hunting/Big-Game-Information/Chronic-Wasting-Disease-CWD; see https://www.ksal.com/chronic-wasting-disease-spreading-in-kansas-deer/.
Kentucky, see https://fw.ky.gov/News/Pages/Chronic-wasting-disease-confirmed-in-Kentucky-for-first-time.aspx.
Louisiana, see https://www.lsu.edu/vetmed/news/2022/laddl_cwd_confirmed.php.
Maryland, see https://dnr.maryland.gov/wildlife/Pages/hunt_trap/CWD_in_Maryland.aspx.
Michigan, see https://www.cidrap.umn.edu/chronic-wasting-disease/michigan-reports-first-cwd-detection-ogemaw-county.
Minnesota, see https://www.dnr.state.mn.us/cwd/about.html.
Mississippi, see https://www.ms-sportsman.com/hunting/deer-hunting/update-on-chronic-wasting-disease-in-mississippi/.
Missouri, see https://www.ozarksfirst.com/news/local-news/which-missouri-counties-showed-deer-with-chronic-wasting-disease/.
Montana, see https://www.greatfallstribune.com/story/news/local/2022/11/11/montana-fwp-confirms-first-case-of-cwd-in-deer-near-belt-montana/69638009007/; see https://www.rmef.org/elk-network/montana-records-first-suspected-case-of-cwd-in-wild-elk/.
Nebraska, see https://outdoornebraska.gov/conservation/conservation-challenges/wildlife-diseases/chronic-wasting-disease/.
New Mexico, see https://www.wildlife.state.nm.us/conservation/invasive-species-and-diseases/chronic-wasting-disease/.
New York, see https://dec.ny.gov/nature/animals-fish-plants/wildlife-health/animal-diseases/chronic-wasting-disease.
North Carolina, see https://www.bowhunting.com/article/first-cwd-case-found-in-north-carolina-deer-herd/.
North Dakota, see https://gf.nd.gov/wildlife/diseases/cwd/faq.
Ohio, see https://ohiodnr.gov/discover-and-learn/safety-conservation/wildlife-management/wildlife-disease/chronic-wasting-disease.
Oklahoma, see https://www.wildlifedepartment.com/hunting/resources/deer/cwd.
Pennsylvania, see https://www.vet.upenn.edu/docs/default-source/research/pa-wildlife-futures-program/threat-assessment-reports/cwd-state-level-dashboard-%281%29.pdf?sfvrsn=d02df6ba_8.
South Dakota, see https://gfp.sd.gov/userdocs/docs/chronic_wasting_disease_faqs.pdf.
Tennessee, see https://www.tn.gov/content/tn/twra/hunting/cwd/cwd-in-tennessee.html#History.
Texas, see https://tpwd.texas.gov/huntwild/wild/diseases/cwd/.
Utah, see https://wildlife.utah.gov/chronic-wasting-disease.html.
Virginia, see https://dwr.virginia.gov/wildlife/diseases/cwd/.
Washington, see https://wdfw.wa.gov/newsroom/news-release/first-chronic-wasting-disease-case-confirmed-spokane-county.
West Virginia, see https://oeps.wv.gov/cwd/Documents/community/cwd_QnA.pdf.
Wisconsin, see https://datcp.wi.gov/Pages/Programs_Services/ChronicWastingDisease.aspx.
Wyoming, see https://wgfd.wyo.gov/Wildlife-in-Wyoming/More-Wildlife/Wildlife-Disease/CWD-in-Wyoming-Wildlife.

While CWD was first detected relatively recently in Texas, there are a relatively high number of locales with endemic CWD. The remaining states all have less than 4000 captive cervids and fewer than 100 farms (Chiavacci, 2022). Both Idaho and Oklahoma have seen a few cases.

For the more eastern states without mule deer, there are regular groupings based on the years since first detection and number of endemic locales, each across the full range of number of cervids and farms. Exceptions to the pattern include Arkansas and Tennessee which both have only recently detected CWD yet have several endemic locations. New York is also different in that CWD was detected 18 years ago, but few other cases have been found. This is fortuitus for New York as the state has almost 7500 captive cervids and over 100 farms (Chiavacci, 2022).

Data were obtained by the committee from a variety of digital sources on state-by-state number of hunting licenses issued annually and populations of wild cervids (the committee realizes these population estimates are rough and do not employ them in a formal analysis, rather as an indication of the magnitude of those populations at risk). Considering deer as the most widespread species at risk, for the same states as employed in Chiavacci (2022) and grouped by states with and without mule deer habitat, Figure F-3 plots the number of hunters in 2022 and wild deer populations in 2022. Figure F-3 illustrates those states that have more deer have more hunters, but the general pattern in the states with mule deer habitat is quite different than those with just white tail deer. It is important to note that there may be significant differences in the benefits and costs of hunting across cervid species and states, and the relative proportions of cervids in states varies significantly–for example many states with mule deer also include significant populations of elk and moose. Also, important to note is there is great variation in access to hunting across states, public lands, and variation in resident and non-resident demand for hunting.



(a) States with mule deer (b) States without mule deer

FIGURE F-3 Relationships between the number of paid hunting license holders in 2022 and wild deer population estimates in 2022. License data are from the U.S. Fish and Wildlife Service (see https://partnerwithapayer.org/funding-sources/). Deer population estimates for 2022 were collected from state agencies and tabulated by https://wildlifeinformer.com/deer-population-by-state/.

Figure F-4 plots the years since first detection and wild deer populations in 2022 in panels (Figure F-4a) and (Figure F-4b), and the number of hunters in 2022, in panels (Figure F-4c) and (Figure F-4d).

The plots illustrate again the differences in the pattern of the data between the western/great plains states and those further east. For the states with mule deer (Figure F-4a and Figure F-4c), both risk factors (long period of time since first detection and number of endemic locals) increase together apart from Montana (with lower numbers of deer and hunters) and Texas (with higher numbers of deer and hunters), where they have several endemic locals given a relatively recent first detection. The same general risk factor pattern persists for states without mule deer Figure F-4b and Figure F-4d), although the groupings of each risk factor combination is maintained across broad ranges of deer populations and number of hunters. Again, outliers are Arkansas and Tennessee (having relatively recent detection and high numbers of endemic locals) and New York, which has only had a few cases of CWD since its initial detection many years ago.

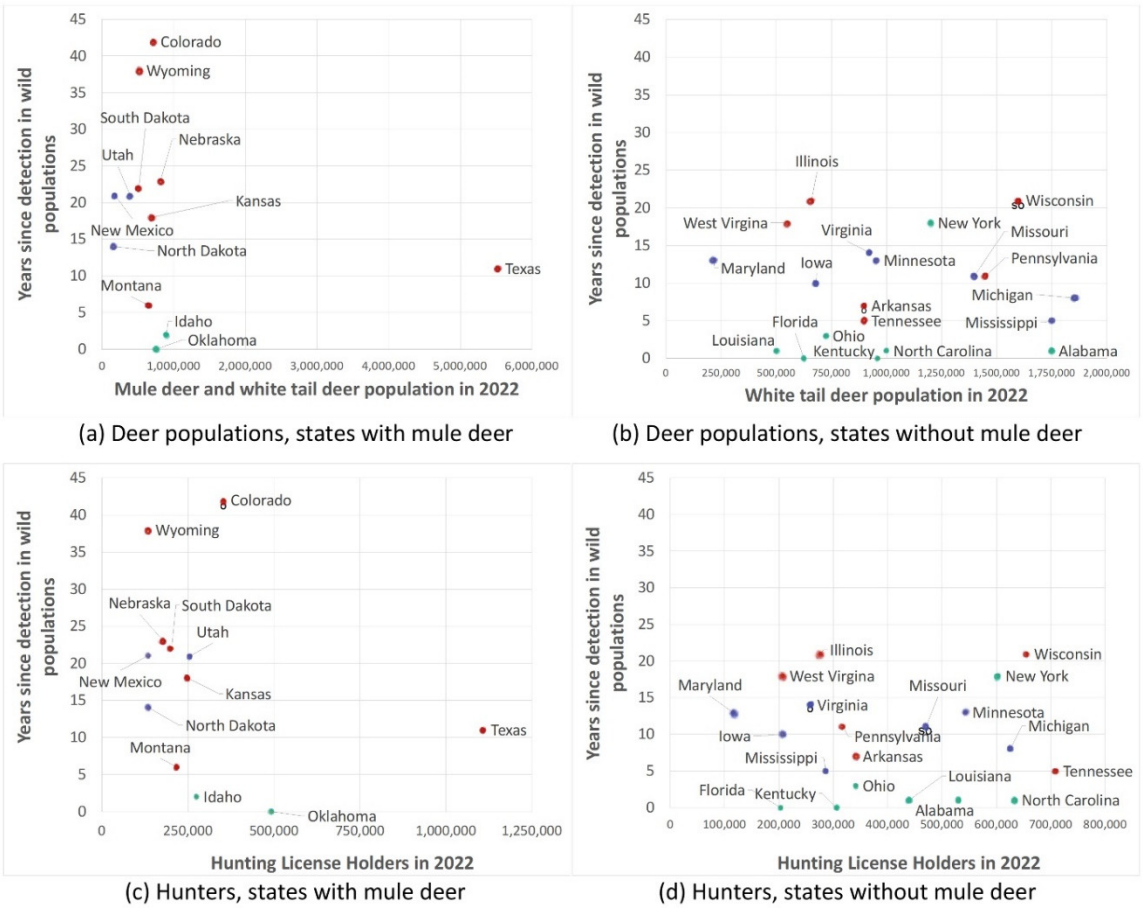


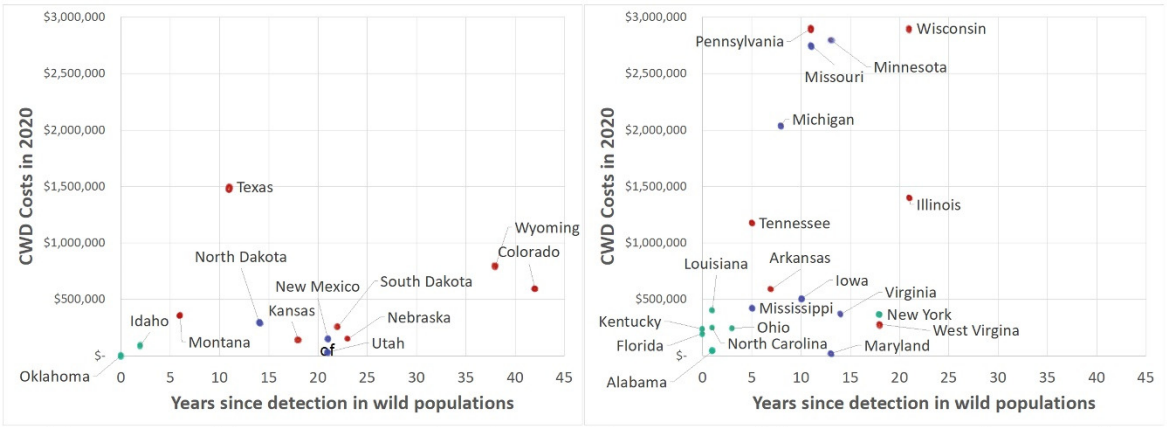
FIGURE F-4 The relationship between years since first detection in wild populations, wild deer population estimates in 2022 and number paid hunting license holders in 2022. The color of dots for each state reflects the classification given by the committee: red dots indicate states with endemic CWD in 20 or more locales; blue dots indicate states with CWD that is endemic in fewer than twenty locales; green dots indicate states with a few cases. Number of years since initial detection are from sources in Footnote 1. License data are from the U.S. Fish and Wildlife Service (see https://partnerwithapayer.org/funding-sources/). Deer population estimates for 2022 were collected from state agencies and tabulated by https://wildlifeinformer.com/deer-population-by-state/.

CWD RELATED COSTS

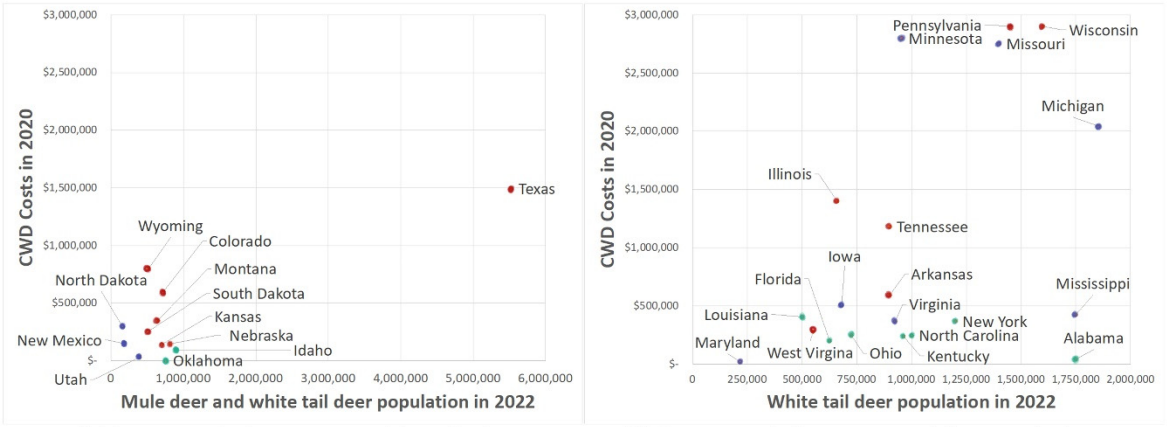
To help understand drivers in public agency costs, the cost data from Table 1 of Chiavacci (2022) was grouped by mule deer habitat and plotted against the number of years since CWD was first detected (Figure F-5a and Figure F-5b); against the population of wild deer (Figure F-5c and Figure F-5d); and against the number of hunting license holders (Figure F-5d and Figure F-5e). As one might expect, on average, states that have experienced more years since the first detection, have high numbers of hunters and high numbers of deer, and tend to have the highest public agency costs from CWD. But the effects depend on whether the states have mule deer habitat or not, and within each grouping there are further differentiations.

For both states with mule deer and without, costs (on average) are higher with years since first detection and more endemic locales (Figure F-5a and Figure F-5b). For the most part, costs are highest in states without mule deer for those which have higher numbers of endemic locales. However, states can have detected CWD relatively recently and have several endemic locales with relatively low costs (Montana, Figure F-5a; Arkansas, Figure F-5b), or high costs (Texas, Figure F-5a; Missouri and

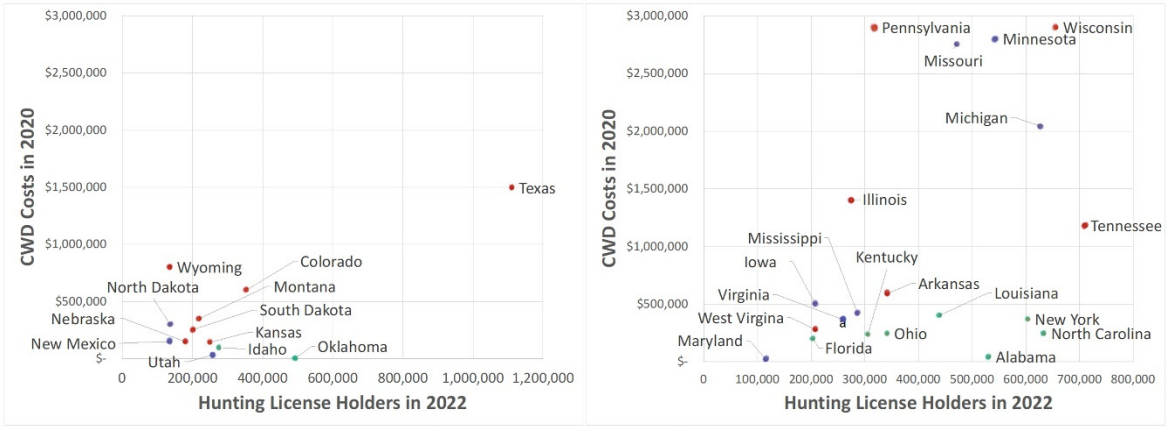
Michigan, Figure F-5b). In addition, there are states which first detected CWD many years ago and have several endemic locales, yet have low costs (Kansas, South Dakota, and Nebraska, Figure F-5a; West Virginia and Maryland, Figure F-5b).



(a) Years since 1st detection, states with mule deer (b) Years since 1st detection, states without mule deer



(c) Deer populations, states with mule deer (d) Deer populations, states without mule deer



(e) Hunters, states with mule deer (f) Hunters, states without mule deer

FIGURE F-5 Relationship between public agency costs (Table 1, Chiavacci, 2022) and: (a, b) number of years since initial detection (Footnote 1); (c, d) number paid hunting license holders (https://partnerwithapayer.org/funding-sources/); and (e, f) total cervid population

(https://wildlifeinformer.com/deer-population-by-state/). Red dots indicate states with endemic CWD in 20 or more locales; blue dots indicate states with CWD that is endemic in fewer than twenty locales; green dots indicate states with a few cases.

The pattern of costs and deer populations or hunters clearly depends on whether, or not, mule deer habitat is in the state. For states with mule deer, costs are higher with higher deer populations or more hunters and more endemic locales. The pattern is less clear in states without mule deer. While costs can be higher for states with more deer or hunters and with more endemic locals, there are wide ranges of deer populations and hunters with the same number of endemic locales and wide ranges of costs. For example, Maryland and West Virginia have lower deer populations and lower numbers of hunters yet moderate to high numbers of endemic locals, and low costs.

REFERENCES

Arnot, C., E. Laate, J. Unterschultz, and W. Adamowicz. 2009. Chronic Wasting Disease (CWD) Potential Economic Impact on Cervid Farming in Alberta. *Journal of Toxicology and Environmental Health.* 72 Part A(17-18):1014-1017. https://doi.org/10.1080/15287390903084223.

Chiavacci, S.J. 2022. The economic costs of chronic wasting disease in the United States. *PLOS ONE* 17(12):e0278366. https://doi.org/10.1371/journal.pone.0278366.

Outlaw J.L., D.P. Anderson, M.L. Earle, and J.W. Richardson. 2017. Economic impact of the Texas deer breeding and hunting operations. Agricultural & Food Policy Center, Department of Agricultural Economics, Texas A&M AgriLife Research, Texas A&M AgriLife Extension Service, Texas A&M University, Research Report 17-3 (College Station, Texas, USA.).