Chronic Wasting Disease Spillover Preparedness and Response:

Charting an Uncertain Future

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Preface

As the burden of chronic wasting disease (CWD) increases among cervids, concerns about prion spillover to other species—including humans and non-cervid production animals—also increase. Despite this, no contingency plans exist at a national or international level to address the possibility of spillover, which would trigger a national and global crisis. Facing this possibility, we are pleased to share the following report, "Chronic Wasting Disease Spillover Preparedness and Response: Charting an Uncertain Future." This effort was supported by a contract from the Minnesota Department of Natural Resources.

Our team was fortunate to collaborate with 67 esteemed experts who shared their diverse knowledge and expertise in human medicine and public health, cervid and production animal health, prion biology and disease diagnostics, carcass and contaminated item disposal and the environment, and wildlife health and conservation during 25 working group meetings. These discussions formed the basis of this report.

We are immensely grateful to all of these collaborators and reviewers, particularly the 10 distinguished co-chairs who led discussions across five working groups and provided additional support to our overall effort. We hope that the resulting recommendations provide useful guidance to professionals in human, animal, and wildlife health agencies; academic researchers; and medical practitioners who will be tasked with responding to a possible human or non-cervid animal CWD spillover incident.

Throughout our working group meetings, we were reminded of a famous quote from US President Dwight D. Eisenhower:

"Plans are nothing, but planning is everything."

This report reflects thousands of hours of planning by many of the best minds working today on CWD.

We realize that the changing landscape of prion biology and disease surveillance complicates any effort to predict a possible spillover incident into humans or non-cervid production animals and the type of response that will be required. As we progressed through this collaborative process, however, it became increasingly clear that there was immense benefit to establishing a network of CWD experts across disciplines, agencies, and countries. We were privileged to learn from them and are confident that their expertise is critical to preparing for and responding to a possible spillover event.

The CIDRAP Chronic Wasting Disease Team

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CIDRAP

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Acronyms

AFWA: Association of Fish & Wildlife Agencies

APHIS: Animal and Plant Health Inspection Service

BAH: Board of Animal Health

BSE: Bovine spongiform encephalopathy

CDC: Centers for Disease Control and Prevention

CFIA: Canadian Food Inspection Agency

CIDRAP: Center for Infectious Disease Research and Policy

CJD: Creutzfeldt-Jakob disease

CNS: Central nervous system

CSF: Cerebrospinal fluid

CWD: Chronic wasting disease

DNR: Department of Natural Resources

ELISA: Enzyme-linked immunosorbent assay

HCP: Herd Certification Program

iCJD: Iatrogenic CJD

IHC: Immunohistochemistry

MRI: Magnetic resonance imaging

NAHLN: National Animal Health Laboratory Network

NASEM: National Academies of Sciences, Engineering, and Medicine

NHP: Nonhuman primate

NPDPSC: National Prion Disease Pathology Surveillance Center

NVSL: National Veterinary Services Laboratory

PHAC: Public Health Agency of Canada

PMCA: Protein misfolding cyclic amplification

Prnp: Gene that encodes for the prion protein in animals.

PRNP: Gene that encodes for the prion protein in humans.

PrP: Prion protein

PrP^{BSE}: Infectious BSE prion protein

PrP^{CWD}: Infectious CWD prion protein

PrP^{C}: Normal cellular prion protein

PrP^{Sc}: Abnormal infectious prion protein

RFID: Radio frequency identification

RPLN: Retropharyngeal lymph node

RAMALT: Rectoanal mucosa-associated lymph tissue

RT-QuIC: Real-time quaking-induced conversion

SAA: Seed amplification assay

sCJD: Sporadic CJD

SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis

vCJD: Variant CJD

TSE: Transmissible spongiform encephalopathy

USDA: US Department of Agriculture

USGS: US Geological Survey

WB: Western blot

WOAH: World Organization for Animal Health

Executive Summary

Chronic wasting disease (CWD) is a fatal neurodegenerative disease of cervids (e.g., white-tailed deer, mule deer, elk, moose, reindeer). It is caused by prions, infectious proteins that target normal brain proteins to fold abnormally. Infected animals shed CWD prions in body fluids, potentially exposing other cervids during social encounters. In addition, shed prions can persist in soil for extended periods and be taken up by plants, leading to environmental contamination and serving as another potential source of infection.

CWD is one of several prion-related transmissible spongiform encephalopathies (TSEs) that occur in animals and humans. Other examples include Creutzfeldt-Jakob disease (CJD), variant CJD (vCJD), and kuru, which occur in humans; bovine spongiform encephalopathy (BSE, or "mad cow disease"), which occurs in cattle and has been transmitted to humans, causing vCJD; and scrapie, which occurs in sheep and goats. CWD was first identified in 1967 in a captive mule deer in Colorado and was found in free-ranging cervids in Colorado and Wyoming in 1981. To date, CWD has been identified in 35 US states, as well as Canada, Finland, Norway, South Korea, and Sweden.

Currently, CWD management is in the domain of wildlife agencies. With no vaccine or treatment available, CWD management focuses on mitigating disease spread. Thus, CWD is likely to become endemic in more areas over time as the agent is introduced to new areas where cervids are present. Because cervids are widespread on every continent except Antarctica, CWD poses a significant global threat.

Two factors, variations in the amino acid coding sequence (polymorphisms) in the host prion protein gene (*Prnp*) and the diversity in properties of CWD strains, influence the risk of CWD in cervids. The interaction between host genetics and CWD strains is complex and still being studied. Because strain properties influence the host range of prion infection, this dynamic strain landscape poses a significant challenge to our ability to predict the risks that CWD prions pose to humans and non-cervid animals.

Given the increasing prevalence of CWD and the evolution of CWD strains, researchers, hunters, and government officials in particular

are increasingly concerned about spillover to production animals or humans. The continued spread of CWD and environmental contamination increase the risk of exposure and spillover to other wildlife, livestock, and humans. Emerging prion strains with a greater propensity for cross-species transmission could heighten these concerns. In addition to human health risks, CWD spillover could have far-reaching effects on the food supply, economy, global trade, and agriculture.

A multidisciplinary approach was needed to assess the current state of CWD and prion disease science, surveillance, and management. This required identifying gaps in spillover preparedness and developing recommendations to improve public and animal health agencies' ability to respond. The Center for Infectious Disease Research and Policy (CIDRAP) identified five key areas within CWD prevention and control: human health, cervid and production animal health, prion biology and disease diagnostics, carcass and contaminated item disposal and the environment, and wildlife health and management. CIDRAP convened five working groups, each led by two co-chairs who are distinguished experts in their respective fields and composed of 57 additional subject matter experts. In this report, we communicate the outputs from the working-group meetings and summarize key findings by subject area.

Key Findings: CWD Management and Surveillance in Wild and Captive Cervids

- Because the management of wild cervid populations generally falls under the jurisdiction of state and provincial governments, approaches to CWD surveillance and management across North America vary significantly both within and between jurisdictions, including tribal jurisdictions, owing to differences in available resources and political willingness to address the disease.
- Wildlife agencies rely on voluntary hunter harvest as the most important mechanism for CWD disease surveillance and management. As a result, agencies are challenged to maintain hunter engagement on this issue. This can be complicated, given the long-term nature of the disease and the perceived burdens that may accompany certain actions, both of which contribute to hunter "disease fatigue."
- At the time of the initial publication of this report, no effective methods are available to eliminate CWD in wild cervid populations.
- Deer, elk, moose, and other cervids are keystone species ecologically and economically, and they are a vital source of protein and a cultural cornerstone for groups such as tribal communities and rural populations. CWD may pose an existential threat to these species and generational hunting traditions.
- Wildlife agency budgets already face constraints because of declining hunting license sales (primarily for deer hunting) and the rising costs of CWD management and surveillance. Documented CWD spillover into humans or non-cervid production animals will exacerbate these funding challenges, underscoring the need for additional support and contingency plans that consider such events.
- While different prion strains can have important implications for disease dynamics, the diversity of CWD strains on the cervid landscape is virtually unknown. Most CWD surveillance programs do not collect strain-level data.
- Surges in demand during annual hunting seasons challenge existing laboratory capacity for CWD testing. Without additional capacity or the availability of less expensive, high-throughput tests, any surge in demand prompted by CWD

spillover will likely overwhelm available testing systems.

- The structures and functions of wildlife agencies are not conducive to proactively addressing big-picture challenges such as potential CWD spillover. Moreover, even with the current reactive approach, maintaining a stable response to a "bad-news issue" such as CWD is complicated by fluctuating political landscapes, changes in agency leadership, waning public interest, and limited resources.
- Because CWD surveillance is not conducted in non-cervid sympatric wild species, the potential for CWD spillover to other wildlife, particularly small mammals, is unknown.
- Despite the potential One Health implications of CWD, wildlife agencies that oversee its management in free-ranging cervids understandably approach the disease exclusively as a wildlife health concern, and the involvement of other agencies such as those involved with public health has been limited. However, if a documented CWD spillover occurs, the role of wildlife agencies would change significantly.
- Federal officials have a greater role in managing CWD in farmed cervids through herd certification programs in the United States and Canada. However, even with such programs in place, CWD continues to spread among farmed cervids in both countries, posing an unquantified but likely growing risk to free-ranging animals.

Key Findings: CWD Diagnostic Laboratory Testing in Animals

- No existing test meets all CWD diagnostic needs because they are context-dependent. Used in tandem, they can provide important information; however, test sensitivity early in disease progression may be low, and important knowledge gaps remain.
- Laboratory capacity is a challenge for CWD testing during the hunting season. Existing validated assays are resource-intensive and require expensive equipment, trained personnel, adequate space, and a sufficient supply of reagents.
- While seed amplification assays (e.g., PMCA, RT-QuIC) have significantly advanced research involving CWD and other prions, universal standardization of protocols and reagents and regulatory validation remain substantive challenges.
- There is uncertainty in distinguishing CWD strains, because strain phenotypes overlap. New developments in strain identification methods will likely result in further refinement of strain definitions and classification.
- Although the implications of emerging CWD strains are relevant to the likelihood of interspecies transmission, available information on CWD strain diversity and related dynamics is limited. One reason for this is the small number of tools that can be used for comprehensive, high-throughput strain typing in surveillance settings.
- Experimental transmission studies provide valuable insight into prion disease species barriers, but the generalizability of results from these models/platforms is limited because laboratory conditions may not mimic natural conditions.
- The inability to predict how CWD would present if transmitted to a non-cervid species generates uncertainty about whether current diagnostic tools are sufficient to recognize spillover within the protocols of existing surveillance systems.
- Although available information strongly suggests that existing amplification assays will be useful in CWD spillover

investigations, whether they or any other diagnostic assays could detect or discriminate between all possible spillover strains is uncertain.

Key Findings: Spillover to Non-Cervid Production Animals: Surveillance, Laboratory Capacity, Planning, and Response

- Prion disease surveillance is limited or absent for most production animals. Although scrapie and BSE surveillance programs are in place for cattle, goats, and sheep, they are insufficient to identify a CWD spillover in all production animals.
- No single test can identify CWD transmission to a non-cervid species, clearly presenting a major diagnostic challenge. In theory, a combination of existing tests could be used, but data are not available on whether they could differentiate BSE or scrapie from CWD in a novel animal host.
- CWD strains likely differ in virulence, transmissibility, and zoonotic potential. However, CWD strains are poorly characterized, agreement on what constitutes a strain is lacking, and easy methods for comprehensive strain typing are not available.
- Following a spillover event in which CWD infects a novel host species, the pathological features of disease (e.g., prion distribution, lymphotropism, prion shedding capacity) could be different than those observed in cervids. Determining these differences is essential to evaluate transmission risk, inform traceback investigations, and determine response strategies (including biosafety guidelines).
- Response to a CWD spillover is complicated because multiple agencies have jurisdictional authority, and detailed contingency plans for interagency cooperation do not exist. Furthermore, regulatory and logistical barriers to access premises for spillover investigations present additional challenges.
- The roles of external academic centers and laboratories during a CWD spillover investigation are undefined, although they can offer essential expertise.
- Regulatory authorities will need to anticipate and plan contingencies for the impact of CWD management on trade policies and production animal and agriculture industries.
- Although precedents exist for agencies to scale up testing in response to disease spillover (e.g., H5N1, COVID-19, bovine tuberculosis), CWD poses unique challenges owing to costs, space demands, and other testing-method unknowns (i.e., some tests are more labor intensive, slower, or unvalidated).

Key Findings: Environmental Implications of Carcass and Contaminated Item Disposal

- CWD prions are highly stable in the environment and can remain infectious for years to decades, withstanding conditions that most other infectious agents cannot. With no effective way to inactivate prions in the environment at scale, the ongoing spread of disease presumably results in ever-increasing accumulation on the landscape.
- CWD prion fate and dynamics in the environment are difficult to characterize because of countless variables that span both the prion agent and the environment (e.g., climate, soil type, strain). Although some research has been conducted on this topic, the ability to conduct experiments in a controlled

environment that mimics real-world conditions is limited.

- An effective, cost-efficient, and scalable disposal system for CWD-infected carcasses and contaminated items that limits environmental contamination has not been established. Each disposal method has notable drawbacks and limitations, and capacity is an issue for all options.
- Approaches to cervid carcass disposal vary across jurisdictions and agencies, as do the related information and communication about these approaches. These outcomes are shaped by factors such as regulatory authority and resource availability.
- Currently, CWD test results from hunter-harvested animals are often not delivered quickly enough to inform the most appropriate and timely disposal options. With an unknown CWD status, the safest approach may be to assume that animals are positive and dispose of them in ways that minimize risk.
- Information is lacking on the effect of carcass disposal measures on reducing the spread of CWD. Agencies rely on individuals to follow the recommended options, but compliance is largely unknown, and the proportion of CWD-positive animals being disposed of properly is unclear. A cost-benefit analysis is needed to assess the effectiveness of current carcass-disposal practices.
- CWD spillover into humans or non-cervid production animals would disrupt the disposal status quo and generate much higher volumes of waste to manage. The current system is not equipped to manage changes in demand.

Key Findings: Detecting CWD Spillover into Humans

- The risk of CWD spillover is not static, and factors such as rising disease prevalence in wildlife and evolving CWD strain characteristics may alter transmissibility to non-cervid species over time. Historical data, therefore, cannot be considered a reliable predictor of current or future spillover risk.
- The zoonotic risk of CWD is not understood in the context of growing exposure, prolonged incubation periods that often accompany interspecies transmission of CWD, or evolving CWD prion strains. Although experimental transmission studies are valuable and inform the understanding of zoonotic risk, extrapolating results and applying them to the real world is difficult because of numerous limitations.
- Physician recognition of prion diseases is limited because these illnesses are rare and have symptoms that overlap with other neurodegenerative diseases. Also, important barriers remain to antemortem testing and diagnosis. Prion disease manifestation in humans can vary by age, prion strain, and other factors that could influence recognition of disease, particularly for patients without access to specialized care.
- Although autopsies are important for investigating and diagnosing prion disease, the number of autopsies completed for this or any other purpose is decreasing, likely due to numerous factors. Coupled with the increasing prevalence of CWD among cervids and the risk of exposure to CWD among humans, the power of the current surveillance system to detect a single case of CWD spillover is limited.
- CWD transmission to a human is uncharacterized, and researchers

have not defined the evidence (clinical, pathological, or epidemiologic) that would identify a spillover incident. Identification would likely depend on the recognition of multiple cases with similar disease features and presumed CWD exposure.

- Obtaining exposure information relevant to prion diseases is challenging, particularly given potentially prolonged incubation periods. Furthermore, widespread gaps in cervid CWD surveillance, outdated consumption estimates for CWD-positive cervids, and extensive but undocumented exposure (i.e., non-hunter venison consumers and environmental exposure) limit the collection of these data.
- CWD spillover-related collaboration among public health, wildlife, and agriculture agencies is somewhat limited. Obstacles include cooperation/agency buy-in, issues related to data privacy (e.g., wildlife agencies sharing hunting license data with public health), funding, and the significant amount of unknowns on the subject.
- Because suspicion of a CWD spillover incident may be met with resistance, skepticism, and fear, pre-scripted messaging and consensus recommendations would be useful in addressing inquiries.

Conclusions and Recommendations

The following recommendations are the result of extensive discussions among experts in 25 working group meetings spanning five topic areas (human health, cervid and production animal health, prion biology and disease diagnostics, carcass and contaminated item disposal and the environment, and wildlife health and management). They highlight and prioritize gaps and needed actions to prepare for the possibility of CWD spillover into other wildlife, non-cervid production animals, or humans.

1. **Advocate for CWD spillover awareness and preparedness among medical providers, research institutions, and wildlife, agricultural, and public health agencies.**
2. **Create sustained, dedicated, multi-year funding sources to support needed efforts.**
3. **Expand and standardize CWD surveillance in wild cervids, other wildlife, and non-cervid production animals to facilitate data summarization and interjurisdictional comparisons.**
4. **Promote human prion disease surveillance among human healthcare providers to enhance capacities to detect CWD spillover to humans.**
5. **Expand CWD research and development efforts.**
6. **Encourage and support formalized interagency and cross-sector collaboration.**
7. **Develop and test proactive CWD spillover communications, messaging, and education.**
8. **Draft regulatory and government agency policies in advance.**
9. **Plan for environmental concerns and estimate disposal capacity needs.**

1. Advocate for CWD spillover awareness and preparedness among medical providers, research institutions, and wildlife, agricultural, and public health agencies.

There is a risk of CWD spillover to other wildlife, non-cervid production animals, and humans. The risk of CWD spillover is not static and may be increasing as new strains emerge and exposure probabilities increase. Historical data may not be a reliable predictor of current or future spillover risk. As a result, there are growing needs for:

- Additional capacity and infrastructure to support CWD research, diagnostic innovation, and preparedness;
- New communication strategies to inform policymakers and stakeholders who would be responsible for a successful CWD spillover identification and response; and,
- Advanced planning, in particular multi-agency tabletop exercises to prepare consistent and coordinated CWD spillover policies and response plans.

2. Create sustained, dedicated, multi-year funding sources to support needed efforts.

Currently available funding is insufficient and largely available only in single-year grants that are poorly designed to meet the concerns raised in this report. As a result, there are increasingly urgent needs to:

- Diversify funding support for state and tribal wildlife agencies so that they can effectively address new interdisciplinary conservation challenges, of which CWD surveillance and response are only one. Hunting license fees, linked excise taxes, and available USDA funding are grossly insufficient and would be incapable of supporting an effective CWD spillover

response. Furthermore, if spillover to humans occurs, deer hunting license sales likely will significantly decrease, compounding budget gaps.

- Build laboratory capacity and develop less expensive, faster, and more sensitive CWD testing to address increases in testing demand following a spillover incident.
- Prioritize research funding (See below: "Expand CWD research and development efforts") to improve early detection and diagnosis of CWD spillover. This should include robust support for basic, hypothesis-driven research that advances scientific understanding and innovation.
- Bolster funding for state, tribal, and federal public health agencies to ensure timely and effective CWD spillover response.
- Educate public health officials and healthcare professionals to improve their understanding of and surveillance for prion diseases.

3. Expand and standardize CWD surveillance in wild cervids, other wildlife, and non-cervid production animals to facilitate data summarization and interjurisdictional comparisons.

Expanded and improved surveillance for CWD is needed in both cervids and targeted non-cervid species to improve the likelihood of detecting potential spillover incidents. Focusing on clinical, pathologic, and epidemiologic evidence will improve surveillance, inform understanding of disease dynamics, and guide public health responses.

- Conduct an assessment of the financial and logistical requirements necessary to improve lab-based surveillance capabilities on a state-by-state basis.
- Systematically expand laboratory capacity to increase sample throughput and decrease testing costs and times so that laboratories can accommodate surges in CWD testing demand (e.g., during hunting seasons).
- Incorporate strain-level data into existing cervid CWD surveillance programs to improve understanding of disease dynamics and management needs.
- Expand and standardize CWD surveillance programs to monitor disease prevalence in both wild cervids and other wildlife species, including targeted non-cervid species that may share habitats with production animals.
- Integrate data from research that tests the natural environment for CWD contamination to optimize agency surveillance protocols.
- Establish provisional criteria for surveillance protocols and identification of CWD spillover incidents among non-cervid wildlife and production animals.

4. Continue and promote human prion disease surveillance, review and refine public health guidance, and educate healthcare providers to enhance capacities to detect CWD spillover.

- Expand the number of longitudinal cohort studies that prospectively follow groups that face greater exposure likelihoods, especially people who frequently consume hunter-harvested deer or elk meat from areas with high CWD prevalence.
- Strengthen coordination between public health agencies, the National Prion Disease Pathology Surveillance Center (NPDPSC), and other laboratories that conduct prion testing of clinical samples from patients with neurodegenerative disease to ensure centralization of US surveillance for human prion disease.
- Develop strategies to promote the consistent collection of detailed and standardized patient histories and risk

factor information for human prion disease cases.

- Continue to raise awareness of the importance of autopsy on suspected cases of human prion disease.
- Formalize public health guidance for scenarios with potential opportunities for exposure to the CWD agent, including testing and basic personal protective equipment recommendations (e.g., wearing gloves when field-dressing a deer carcass).
- Enhance physician awareness of (1) human prion disease, with a focus on the potential risk of CWD spillover from consumption of CWD-contaminated meat, particularly in areas with high CWD prevalence in cervids, (2) location-specific reporting requirements of prion disease cases, and (3) the diagnostic services that NPDPSC provides to confirm human prion disease in suspected cases.
- Obtain updated population statistics on hunters and dietary habits regarding CWD risk factors.

5. Expand CWD research and development efforts.

There is a pressing need to better understand evolving CWD prion strain characteristics and associated spillover risk.

- Conduct additional large-animal studies to assess the risk of interspecies transmission and document clinical signs, tissue distribution, and shedding potential of CWD prions in non-cervid hosts infected via the oral-nasal route. Additional facilities capable of housing large animals for research are necessary to fill this gap.
- Develop and integrate accessible strain-typing technology into CWD surveillance protocols to investigate strain properties as a contributing factor to transmissibility and virulence.
- Support the innovation and delivery of new rapid (and antemortem) diagnostic tests that can identify CWD in cervids and non-cervid species and support surge testing needs.
- Accelerate the development and validation of rapid (and antemortem) diagnostic tests.
- Refine understanding of the dynamics and infectivity of CWD prions in environmental media (e.g., soil, water, vegetation) through optimized research methods.
- Identify factors that influence CWD prion persistence in environmental media and test potential mitigation strategies.
- Improve understanding of interspecies and environmental transmission risks.
- Support and expand the tissue repository under development at the National Wildlife Research Center.
- Expand human dimensions studies related to CWD.

6. Encourage and support formalized interagency and cross-sector collaboration.

Proactive collaboration among agencies that regulate wildlife, public health, agriculture, and the environment is needed to develop integrated CWD management strategies, including those that address spillover risks.

- Formalize and foster relationships across local, state, regional, tribal, and federal agencies to facilitate CWD coordination, management, and response.
- Collaborate to develop interagency CWD spillover contingency plans that can be included in state CWD response plans, clarify roles and responsibilities, and organize communication and response efforts.

- Foster interdisciplinary collaborations among government agencies, researchers, public health officials, wildlife managers, tribal nations, veterinarians, and physicians to facilitate sharing of knowledge, data, resources, and best practices related to prion disease research, CWD surveillance, and spillover risk and response.
- Obtain required laboratory permits in advance to enable non-governmental (e.g., academic) experts to contribute to spillover investigations.
- Establish a scientific advisory group of prion biology, neurology, CWD, and policy experts to support and advise government agency investigations of apparent CWD spillover incidents, including providing assessments of and recommended responses to the associated public health risk as needed.

7. Develop and test proactive CWD spillover communications, messaging, and education.

Government agencies should consider preparing messaging and language in advance to address various levels of public health risk posed by a CWD spillover incident under investigation. Different approaches may be needed for different audiences.

- Align messaging efforts with other emerging-disease communications, focusing on the facts to help maintain trust early in a CWD spillover investigation.
- Develop separate educational messaging for targeted professionals (e.g., healthcare providers, veterinarians, wildlife personnel) about prion diseases in general, and CWD specifically, to improve understanding and recognition of prion diseases and potential CWD spillover cases. This may highlight clinical signs that overlap between identified spillover cases and other neurodegenerative diseases of a respective species.
- Develop pre-scripted messaging and consensus recommendations for the general public to address anticipated public inquiries about CWD spillover risks, anticipating skepticism and fear and fostering trust through transparent communication.
- Identify innovative methods to engage different audiences with spillover communications to ensure the receipt of information, including those with "disease fatigue" and different generations that may obtain information differently.
- Consider initiatives to counteract CWD-related misinformation on various platforms.

8. Draft regulatory and government agency policies in advance.

- To the extent possible, policies and data use agreements should be drafted proactively, periodically reviewed and updated, and available should a spillover occur.
- Develop general recommendations for a spillover testing protocol for federally regulated diagnostic laboratories. Specific recommendations may vary by species or location.
- Continue to pursue validation of prion seeding amplification assays to facilitate their acceptance and use in diagnostic investigations.
- Draft policies that prioritize ecosystem health and data-driven actions for potential spillover response by state wildlife agencies, including guidance for targeted cervid surveillance and any anticipated changes to hunting activities. These policies should be developed in collaboration with the National Fish and Wildlife Health Initiative steering committee, which includes personnel

from state and federal wildlife agencies, the USDA, and tribal nations.

- Prepare and expedite data use agreements or memoranda of understanding so data may be shared between different agencies and, possibly, other stakeholders while investigating a potential CWD spillover incident.
- Continue to promote the use of electronic tagging systems in all facilities housing captive cervids and across animal agriculture to improve animal traceability and aid in potential spillover investigations.

9. Plan for environmental concerns and estimate disposal capacity needs.

Assessments of the impact of disposal activities on the environmental burden of CWD and disease outcomes, as well as disposal capacity needs, are necessary to adapt and refine disposal strategies over time should a spillover occur.

- Establish long-term monitoring programs to assess the environmental impact of current carcass disposal practices and CWD prion dynamics.
- Allocate state and federal resources to strengthen capacity for disposal of CWD-infected carcasses and other contaminated materials as a precaution for CWD spillover into non-cervid production animals. Groups of neighboring states could work together to address disposal needs and share large facilities and associated costs.
- Standardize protocols for carcass and contaminated-item disposal across jurisdictions to ensure consistency and efficiency, considering local environmental conditions and prion stability.
- Develop innovative, cost-effective technologies for disposal of CWD-infected cervid and non-cervid production animal carcasses.
- Comprehensive cost-benefit analyses of current carcass disposal methods to evaluate their effectiveness in preventing CWD spread are also needed to inform policy changes and resource allocation.

Introduction

The Public Health Risk of CWD

Overview

Chronic wasting disease (CWD) is a fatal neurodegenerative disease of cervids such as white-tailed deer, mule deer, elk, moose, and reindeer. It is caused by infectious misfolded proteins known as prions, which—upon entry into susceptible hosts—can target normal prion proteins throughout the body and induce further misfolding. CWD is one of several prion-related transmissible spongiform encephalopathies (TSEs) named for the characteristic sponge-like appearance of brain tissue observed in infected animals and humans (Bartz 2024). Ongoing accumulation of abnormal, converted prions leads to neuronal loss, resulting in spongiform pathology, neurological decline, and eventually, death. Notably, CWD is the only prion disease that has been detected in free-ranging wildlife (Kurt 2016), which has resulted in numerous disease-management challenges and complexities. Examples of other prion diseases are Creutzfeldt-Jakob disease (CJD), variant CJD (vCJD), and kuru, which occur in humans; bovine spongiform encephalopathy (BSE, or "mad cow disease"), which occurs in cattle and has transmitted to humans, causing vCJD; and scrapie, which occurs in sheep and goats. This introductory chapter explores the characteristics and dynamics of CWD in its natural hosts, summarizes the risk of spillover into non-cervid species, and outlines how this project addresses knowledge gaps while raising awareness on the subject.

CWD in Cervids

CWD prions are introduced into a host through the alimentary (gastrointestinal) tract or possibly, via exposure to other mucosal surfaces (e.g., nasal, ocular). Ingested or inhaled prions are likely transported across epithelial cells into lymph that enters the bloodstream and infects lymphoid tissues such as lymph nodes. This allows prions to enter the peripheral nervous system and infect central nervous system tissues. Infectious prions convert normal prion proteins into misfolded isoforms that accumulate in the brain and other infected tissues. CWD prions accumulate in the central nervous system and other tissues of infected animals, leading to neurological signs such as behavioral changes and weight loss, with death commonly occurring an estimated 18 months to 2 years after infection (Haley 2014, Rivera 2019, WOAH 2019). This established disease progression is the reason

for diagnosing CWD through sampling the retropharyngeal lymph nodes (RPLN) and obex of the brain.

Infected animals shed CWD prions in body fluids such as saliva, semen, urine, feces, antler velvet, and blood throughout the course of illness (Angers 2009, Denkers 2020, Denkers 2024), which can expose other cervids during frequent social encounters (Henderson 2020). In addition, shed prions retain infectivity in soil for long periods and can be taken up by plants, leading to environmental contamination, which can be another source of infection for at-risk cervids (Carlson 2023, Pritzkow 2015). Vertical transmission from infected mothers to newborns has also been identified (Nalls 2013, Selariu 2015). Currently, the relative contributions of vertical, direct (cervid-to-cervid), and indirect (environmental contamination) transmission to disease spread remain unclear, and researchers do not know if they may change over time. Although precise dose-response relationships are unknown, the threshold of prion exposure required to trigger prion amplification and disease progression in cervids appears to be very low (Denkers 2020).

CWD was first identified in 1967 in Colorado in a captive mule deer living in an animal research facility (Williams 1980). Wildlife officials subsequently identified the disease in free-ranging cervids in Colorado and Wyoming in 1981 (Spraker 1997, Miller 2000). Since then, CWD has been identified elsewhere in the United States and in Canada, Finland, Norway, South Korea, and Sweden (CDC 2024). In 2000, CWD was reported in captive or free-ranging cervids in just five US states and one Canadian province but has now been detected in free-ranging cervids in 35 US states and four Canadian provinces and in captive cervid facilities in 20 states and three provinces (Figure 1; USGS 2024). The disease is highly endemic in some areas of North America, including northeastern Colorado, southeastern Wyoming, and southwestern

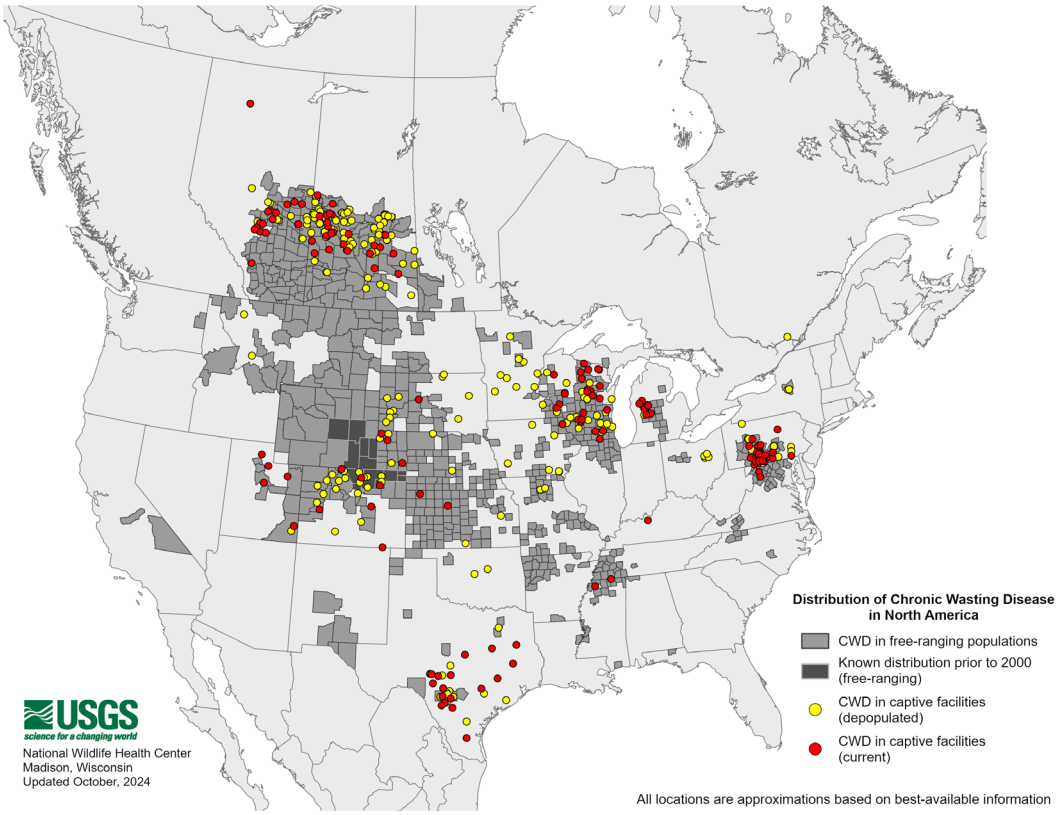


Figure 1: Distribution of Chronic Wasting Disease in North America, Updated December 2024.

For the most recent distribution, see: US Geological Survey

Wisconsin in the United States and in Alberta and Saskatchewan in Canada. In parts of southwestern Wisconsin, 40% to 50% of adult male white-tailed deer are positive for CWD (WI DNR), and in southern Saskatchewan, the prevalence among male mule deer is as high as 80% (Gilch 2022). The increasing prevalence and distribution of CWD among wild cervids stems from prions' high transmissibility and prolonged stability in the environment, among other factors.

Documented CWD cases in Scandinavia and South Korea underscore the international threat of CWD to global cervid populations. While CWD was likely introduced into South Korea via elk imported from Canada (Kim 2005), current evidence indicates that Scandinavian CWD emerged independently of North American CWD (Nonno 2020). With no available vaccine or treatment, management of CWD, particularly in free-ranging cervids, is focused on mitigation, with the aim of slowing (but not stopping) disease spread. Thus, CWD is likely to become endemic in more areas as the agent is introduced to new areas where cervids are present. Because cervids are widespread on every continent except Antarctica, the potential global impact of CWD is significant.

Two main factors, variations in the amino acid coding sequence (polymorphisms) of the host prion protein gene (Angers 2010, Hannaoui 2021, Alam 2024) and the strain properties of the infecting CWD prion (Carta 2022, Otero 2022, Pritzkow 2022) influence the risk profile of CWD . While the exact mechanisms by which host genetics and CWD strains interact are complex and continue to be studied (Bartz 2024), research has demonstrated that *Prnp* genetics affect the rate of CWD progression such that some animals develop clinical signs relatively rapidly (Moazami-Gourdarzi 2021), while others exhibit prolonged incubation periods in which they are infected with CWD without showing clinical signs (Hannaoui 2021). However, no combination of genetic determinants encodes complete resistance to CWD infection; all CWD-infected animals eventually succumb to their illness.

The strain properties of CWD prions control many aspects of disease, including the rate at which host prion protein (PrP^{C}) is converted to its pathogenic counterpart, PrP^{Sc}. CWD prion strain properties also influence the shedding profile of prions during the asymptomatic phase of disease and the time that prions remain infectious in the environment (Bartz 2021), factors influencing the widespread transmission of CWD prions in North American cervids. Strain properties also control the host range of prion infection, which in turn is influenced by host *Prnp* genetics. For example, the properties of CWD prions derived from white-tailed deer differ from those prevalent in elk (Race 2007). Conversely, genetic variations in *Prnp* may also influence the emergence of novel strains in a newly infected host (Bartz 2021). CWD prion strains may undergo adaptation in response to various selective pressures (Bian 2021), including the route of infection (DeFranco 2024); these changes can either be maintained or further modified upon passage to a new host (Block 2022, Saunders 2012).

While drawbacks in our ability to rapidly and accurately characterize prion strain properties currently limit our capacity to assess the true prevalence of CWD prion strains in free-ranging cervids across North America, evidence suggests that novel strains will continue to emerge and evolve over time. Support for this hypothesis comes from studies comparing the properties of CWD prions from North America and newly emergent cases from Norway, Sweden, and Finland. These studies demonstrate that the strain properties of North American and Nordic CWD prions are distinct, suggesting that CWD was not introduced into Nordic countries from North America (Bian 2021, Sun 2023, Nonno 2020). The distinct properties of emergent Nordic CWD prion strains may also suggest a sporadic etiology that to date has not been observed in North America. At the time of this publication, research groups have

identified at least 10 CWD strains, a number likely to increase as novel strains continue to emerge and advances are made in prion strain typing (Otero 2022). Because strain properties influence the host range of prion infection, this dynamic strain landscape poses a significant challenge to our ability to predict the risks that CWD prions pose to humans and non-cervid animals.

Different groups are developing vaccines to reduce the likelihood of CWD in animals, but the lack of host immune response to prions is challenging. Researchers are exploring novel approaches, with progress in the identification of protective vaccine components (Napper 2023). However, many wildlife practitioners are skeptical of achieving long-term control of CWD in wild cervids through vaccination because of mobile populations, prolonged environmental persistence, and wide CWD geographic range. Vaccine delivery to free-ranging populations also would be a significant obstacle.

Risk of CWD Spillover

As the prevalence of CWD increases in cervid populations, the likelihood of encountering an infected animal or prion-contaminated environment inherently increases for all species sharing the affected ecosystem. The continued spread of CWD, particularly across North America, and the associated environmental contamination increase the risk of CWD exposure and potential spillover to other wildlife, livestock, and humans. Several studies suggest possible susceptibility to CWD of other wildlife species such as feral swine, raccoons, and several species of North American rodents (Heisey 2010, Cassmann 2022, Moore 2022, Soto 2025). These animals could act as reservoir species owing to broad habitat overlap with cervids (Escobar 2020). However, surveillance measures are not in place to detect spillover to wildlife species that share the same ecosystem as cervids.

As CWD continues to spread, additional prion strains with potentially greater propensity to impact species transmission may emerge (Hannaoui 2017). Animal studies demonstrate that the method of infectious-prion delivery and passage through a known susceptible species alters the CWD prion strain, which in turn can alter transmission potential within and between species (Block 2022, Saunders 2012). Transmission of CWD to non-cervid production animals is of particular concern because it could lead to contamination of a local, regional, or globally distributed food supply. Investigating strain diversity and evolution is a primary focus of current research, as evolving prion strains pose unknown risks and could lead to greater spread within cervids and transmission across the species barrier to other animals or humans.

The risk of animal prion disease spillover and consequences to human health are not just theoretical, as evidenced by the BSE crisis that occurred in the United Kingdom in the 1980s and 1990s. This outbreak led to the diagnosis of 233 human vCJD cases—many in young, previously healthy people—and thousands more suspected infections worldwide linked to consumption of contaminated food products from classical BSE-infected animals (Ward 2006). Cases of atypical BSE, which occurs naturally and sporadically in cattle at very low frequency, were identified in the early 2000s as the result of enhanced surveillance (WOAH 2023). In addition, classical or atypical BSE caused prion diseases in four other animal species through natural and experimental transmission. These include exotic ungulate encephalopathy, feline spongiform encephalopathy, transmission to non-human primates in France, and transmissible mink encephalopathy (Jeffrey 1988, Aldhous 1990, Bons 1999, Baron 2007).

As the CWD burden increases, spillover concerns to humans also increase. CWD researchers, hunters, and government officials are especially vigilant. The implications of long-term human exposure to CWD prions and the disease manifestation in non-cervid animals and humans are unknown. Although the cervid-to-human species barrier appears

strong, that could change over time as new prion strains emerge; therefore, it is prudent to assess the potential risk of spillover incidents and develop contingency plans now.

Improving Prevention of and Response to CWD Spillover

Rationale for Action

Current CWD response efforts are constrained by limited resources and inconsistent disease monitoring across North American regions. In addition, several aforementioned factors suggest that the risk of CWD spillover incidents, particularly in North America, may escalate in the near future. The disease is spreading relatively quickly across North America, leading to increasing areas of endemicity, which may in turn result in a higher prevalence of infection and greater environmental contamination, although these metrics are difficult to measure because of the uneven intensity of surveillance among jurisdictions (Ruder 2024). A consensus study conducted by the National Academies of Sciences, Engineering, and Medicine (NASEM) reviewed the state of knowledge of CWD in US cervid populations to inform future CWD management strategies in cervids (NASEM 2024). Notably, increasing disease prevalence necessarily increases the likelihood of human exposures and provides greater opportunity for prion strain evolution that could lead to changes that alter the species barrier, making cross-species transmission to food-producing animals or humans more possible. This report (*Chronic Wasting Disease Spillover Preparedness and Response: Charting an Uncertain Future*) provides a focused discussion of spillover risks and actions needed now to reduce the health and economic implications of a possible spillover.

In July 2023, the Center for Infectious Disease Research and Policy (CIDRAP) at the University of Minnesota, supported by a contract with the Minnesota Department of Natural Resources (Contract #238845), embarked on a process to: (1) identify key gaps in preparedness for CWD spillover events and; (2) develop a set of recommendations to improve the ability of public health and animal health (including livestock and wildlife) agencies to detect and respond to such events. This report represents a multidisciplinary effort to define key issues that can inform contingency planning for possible CWD detection outside of its natural hosts. The findings and conclusions within the report do not necessarily reflect the positions or policies of individuals or their respective agencies.

Project Methodology: A Scenario-Based Approach

At the outset of this project, CIDRAP identified five areas of focus within the broad field of CWD prevention and control: human medicine and public health; cervid and production animal health; prion biology and disease diagnostics; carcass and contaminated item disposal and the environment; and wildlife health and conservation. To address these focus areas, CIDRAP staff assembled and convened five working groups, each with two co-chairs who are distinguished experts in their respective fields. Sixty-seven subject matter experts participated in working group discussions (Appendix A). Each working group met virtually five times over the course of the project (November 2023 to August 2024).

The working group meetings operated under Chatham House Rules—insights and information shared were not attributed to a single person or institution. This approach allowed participants to respond and ask questions without being "on the record." The CIDRAP team captured key findings via anonymized written notes for each meeting and disseminated them to the corresponding working group members for their future reference and to inform those who were unable to attend the meeting.

Each working group was asked to consider three different scenarios. The first scenario was the status quo, in which no spillover

events were identified. For this scenario, participants had the opportunity to describe their experiences with the current state of CWD science, management, surveillance, and epidemiology, while also helping identify key gaps in knowledge needing additional research. The other two scenarios involved hypothetical events of CWD spillover into either non-cervid production animals such as cattle or into humans. Insights across all working group topics were critical to more fully characterize plans that may be in place now but also were highly useful in identifying gaps and needs for future contingency planning for CWD spillover. In this report, we present outputs from the working group meetings, and each chapter identifies key findings for the five topic areas. Information in the report, unless otherwise cited, was sourced from one or more working group discussions. The final chapters for this report are (in order): (1) CWD management and surveillance in wild and captive cervids; (2) prion diagnostics; (3) spillover to non-cervid production animals: surveillance, laboratory capacity, planning, and response; (4) environmental implications of carcass and contaminated item disposal; and (5) detection of CWD spillover into humans.

The next phase of CIDRAP's CWD Program will shift to sharing working group findings with interested and affected groups through webinars, conference presentations, and publications. In addition to outreach, targeted preparedness activities will include organizing discussion-based exercises involving hypothetical scenarios at the state and national level to further define key steps in contingency planning for CWD spillover to production animals or humans. Engaging local, state, and federal agency partners in these exercises will be key to identifying roles and responsibilities for spillover response. Findings from these exercises will be disseminated to stakeholders and organizations that may be involved in the emergency response to a CWD spillover incident. As further outreach, education, and preparedness efforts occur, this report will be treated as a living document and updated periodically to reflect new developments in the field.

References

Alam P, Hoyt F, Artikis E, et al. Cryo-EM structure of a natural prion: chronic wasting disease fibrils from deer. Acta Neuropathol. 2024 Oct 24;148(1):56 [Full text]

Aldhous P. Spongiform encephalopathy found in cat. Nature. 1990 May 17;194:345 [Full text]

Angers RC, Kang HE, Napier D, et al. Prion strain mutation determined by prion protein conformational compatibility and primary structure. Science. 2010 May 28;328(5982):1154-8 [Full text]

Angers RC, Seward TS, Napier D, et al. Chronic wasting disease prions in elk antler velvet. Emerg Infect Dis. 2009 May;15(5):696-703 [Full text]

Baron T, Bencsik A, Biacabe AG, et al. Phenotypic similarity of transmissible mink encephalopathy in cattle and L-type bovine spongiform encephalopathy in a mouse model. Emerg Infect Dis. 2007 Dec;13(12):1887-94 [Full text]

Bartz JC. Environmental and host factors that contribute to prion strain evolution. Acta Neuropathol. 2021 Jul;142(1):5-16. doi: 10.1007/s00401-021-02310-6 [Full text]

Bartz JC, Benavente R, Caughey B, et al. Chronic wasting disease: State of the science. Pathogens. 2024 Feb 2;13(2):138 [Full text]

Bian J, Kim S, Kane SJ, et al. 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 deer prion protein. PLoS Pathog. 2021 Jul 26;17(7):e1009748 [Full text]

Block AJ, Bartz JC. Prion strains: Shining new light on old concepts. Cell Tissue Res. 2022 Jul 7;392(1):113-33 [Full text]

Bons N, Mestre-Frances N, Belli P, et al. Natural and experimental oral infection of nonhuman primates by bovine spongiform encephalopathy agents. Proc Natl Acad Sci. 1999 Mar 30;96(7):4046-51 [Full text]

Carlson CM, Thomas S, Keating MW, et al. Plants as vectors for environmental prion transmission. iScience. 2023 Nov 10;26(12):108428 [Full text]

Carta M, Aguzzi A. Molecular foundations of prion strain diversity. Curr Opin Neurobiol. 2022 Feb;72:22-31 [Full text]

Cassmann ED, Frese AJ, Moore SJ, et al. Transmission of raccoon-passaged chronic wasting disease agent to white-tailed deer. Viruses. 2022 Jul 20;14(7):1578 [Full text]

Centers for Disease Control and Prevention (CDC). About Chronic Wasting Disease (CWD). 2024 Apr 17 [Web page]

DeFranco JP, Bian J, Kim S, et al. Propagation of distinct CWD prion strains during peripheral and intracerebral challenges of gene-targeted mice. Proc Natl Acad Sci USA. 2024 Aug 6;121(32):e2402726121 [Full text]

Denkers ND, Hoover CE, Davenport KA, et al. Very low oral exposure to prions of brain or saliva origin can transmit chronic wasting disease. PLoS One. 2020 Aug 20;16(6):e0253356 [Full text]

Denkers ND, McNulty EE, Kraft CN, et al. Temporal Characterization of Prion Shedding in Secreta of White-Tailed Deer in Longitudinal Study of Chronic Wasting Disease, United States. Emerg Infect Dis. 2024 Oct;30(10):2118-2127 [Full text]

Escobar LE, Pritzkow S, Winter SN, et al. The ecology of chronic wasting disease in wildlife. Biol Rev Camb Philos Soc. 2020 Apr;95(2):393-408 [Full text]

Gilch S. Chronic wasting disease—A prion disease through a One Health lens. Can Vet J. 2022 Apr;63(4):431-3 [Full text]

Haley NJ, Hoover EA. Chronic wasting disease of cervids: Current knowledge and future

perspectives. Annu Rev Anim Biosci. 2014 Oct 2;3:305-25 [Full text]

Hannaoui S, Schatzl HM, Gilch S. Chronic wasting disease: Emerging prions and their potential risk. PLoS Pathog. 2017 Nov 2;13(11):e1006619 [Full text]

Hannaoui S, Triscott E, Duque Velásquez C, et al. New and distinct chronic wasting disease strains associated with cervid polymorphism at codon 116 of the *Prnp* gene. PLoS Pathog. 2021 Jul 26;17(7):e1009795 [Full text]

Heisey DM, Mickelsen NA, Schneider JR, et al. Chronic wasting disease (CWD) susceptibility of several North American rodents that are sympatric with cervid CWD epidemics. J Virol. 2010 Jan;84(1):210-5 [Full text]

Henderson DM, Denkers ND, Hoover CE, et al. Progression of chronic wasting disease in white-tailed deer analyzed by serial biopsy RT-QuIC and immunohistochemistry. PLoS One. 2020 Feb 14;15(2):e0228327 [Full text]

Jeffrey M, Wells GAH. Spongiform encephalopathy in a nyala (*Tragelaphus angasi*). Vet Pathol. 1988 Sep;25(5):398-9 [Full text]

Kim TY, Shon HJ, Joo YS, et al. Additional cases of chronic wasting disease in imported deer in Korea. J Vet Med Sci. 2005 Aug;67(8):753-9 [Full text]

Kurt TD, Sigurdson CJ. Cross-species transmission of CWD prions. Prion. 2016;10(1):83-91 [Full text]

Miller MW, Williams ES, McCarty CW, et al. Epizootiology of chronic wasting disease In free-ranging cervids In Colorado and Wyoming. J Wildl Dis. 2000 Oct;36(4):676-90 [Full text]

Moazami-Goudarzi K, Andréoletti O, Vilotte JL, et al. Review on *PRNP* genetics and susceptibility to chronic wasting disease of Cervidae. Vet Res. 2021 Oct 7;52(1):128 [Full text]

Moore SJ, Carlson CM, Schneider JR, et al. Increased attack rates and decreased incubation periods in raccoons with chronic wasting disease passaged through meadow voles. Emerg Infect Dis. 2022 Apr;28(4):793-801 [Full text]

Nalls AV, McNulty E, Powers J, et al. Mother to offspring transmission of chronic wasting disease in Reeves' muntjac deer. PLoS One. 2013 Aug 14;8(8):e71844 [Full text]

Napper S, Schatzl HM. Oral vaccination as a potential strategy to manage chronic wasting disease in wild cervid populations. Front Immunol. 2023 Apr 14;14:1156451 [Full text]

National Academies of Sciences, Engineering, and Medicine. State of Knowledge Regarding Transmission, Spread, and Management of Chronic Wasting Disease in U.S. Captive and Free-Ranging Cervid Populations. The National Academies Press. 2024. [Full text]

Nonno R, Di Bari MA, Pirisinu L, et al. Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America. Proc Natl Acad Sci USA. 2020 Dec 8;117(49):31417-26 [Full text]

Otero A, Duque Velásquez C, McKenzie D, et al. Emergence of CWD strains. Cell Tissue Res. 2022 Oct 6;392(1):135-48 [Full text]

Pritzkow S. Transmission, strain diversity, and zoonotic potential of chronic wasting disease. Viruses. 2022 Jun 25;14(7):1390 [Full text]

Pritzkow S, Morales R, Moda F, et al. Grass plants bind, retain, uptake and transport infectious prions. Cell Rep. 2015 May 14;11(8):1168-75 [Full text]

Race BL, Meade-White KD, Ward A, et al. Levels of abnormal prion protein in deer and elk with chronic wasting disease. Emerg Infect Dis. 2007 Jun;13(6):824-30 [Full text]

Rivera NA, Brandt AL, Novakofski JE, et al. Chronic wasting disease in cervids: Prevalence, impact and management strategies. Vet Med (Auckl). 2019 Oct 2;10:123-39 [Full text]

Ruder MG, Fischer JR, Miller MW. Reinterpreting chronic wasting disease emergence in the USA in light of historical surveillance limitations. J Wildl Dis. 2024 Oct 15 [Full text]

Saunders SE, Bartelt-Hunt SL, Bartz JC. Occurrence, transmission, and zoonotic potential of chronic wasting disease. Emerg Infect Dis. 2012;18(3):369-76 [Full text]

Selariu A, Powers JG, Nalls A, et al. *In utero* transmission and tissue distribution of chronic wasting disease-associated prions in free-ranging Rocky Mountain elk. J Gen Virol. 2015 Nov;96(Pt 11):3444-55 [Full text]

Soto P, Bravo-Risi F, Benavente R, et al. Detection of prions in wild pigs (*Sus scrofa*) from areas with reported chronic wasting disease cases, United States. Emerg Infect Dis. 2025 Jan;31(1) [Full text]

Spraker TR, Miller MW, Williams ES, et al. Spongiform encephalopathy in free-ranging mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*) in north central Colorado. J Wildl Dis. 1997 Jan;33(1):1-6 [Full text]

Sun JL, Telling GC. New developments in prion disease research using genetically modified mouse models. Cell Tissue Res. 2023 Apr;392(1):33-46 [Full text]

United States Geological Survey (USGS). Distribution of Chronic Wasting Disease in North America (Updated). 2024 [Web page]

Ward HJ, Everington D, Cousens SN, et al. Risk factors for variant Creutzfeldt-Jakob disease: A case-control study. Ann Neurol. 2006 Jan;59(1):111-20 [Full text]

Williams ES, Young S. Chronic wasting disease of captive mule deer: A spongiform encephalopathy. J Wildl Dis. 1980 Jan;16(1):89-98 [Full text]

Wisconsin Department of Natural Resources. CWD Prevalence in Wisconsin [Web page]

World Organization for Animal Health. Bovine Spongiform Encephalopathy. 2023 [Web page]

World Organization for Animal Health. Technical Disease Card: Chronic Wasting Disease. 2019 [Full text]

Chapter 1: CWD Management and Surveillance in Wild and Captive Cervids

Introduction

This chapter provides an overview of current CWD surveillance and management practices for both wild and captive cervids in the United States and Canada. It also identifies the most important challenges that impede effective disease suppression and considers ongoing and future implications of CWD's expanding distribution and increasing prevalence for wildlife health and conservation.

State, Provincial, and Tribal Agency CWD Surveillance and Responses

Overall Challenges for Wildlife Agencies in Responding to CWD

In the United States and Canada, management and surveillance of CWD in free-ranging cervids is under the jurisdiction of state and provincial wildlife agencies. These agencies face several challenges that are highly relevant to CWD and complicate disease response. The most important of these challenges are:

- Most state wildlife agencies rely on hunting license sales for most of their funding, and deer and elk hunting is the cornerstone of that "user-pay" model. Of the 11.4 million Americans who hunt, 77% pursue deer or elk (USFWS 2018). Given the immense value of wild cervids as a natural and economic resource, CWD management and surveillance are top priorities of most wildlife agencies (Thompson 2022). However, these activities are costly. For example, in 2020, US state wildlife agencies collectively spent more than $25.5 million on CWD-related work, and that number has likely grown over the past few years (Chiavacci 2022). Notably, agencies in states with CWD spent more than eight times more than agencies in states without documented cases, demonstrating both the steep costs that accompany spread of the disease into a new area and the limited investment in management activities prior to detection. CWD can significantly affect the financial capacity of wildlife agencies by further constraining already limited budgets and

potentially diverting funds from other important conservation activities.

- There is no standard approach to CWD surveillance and management. Instead, relevant biological elements (e.g., wildlife population dynamics, habitats) and sociopolitical influences (e.g., available funding, political will, public acceptance) shape agency activities. Cooperative efforts such as the CWD Research Consortium, a voluntary partnership of agency and university specialists, promote multijurisdictional collaboration to address strategic and collective objectives, although such organizations have no regulatory authority and operate at the discretion of employer agencies (CWD Research Consortium).
- CWD management is complicated by prions' persistent infectiousness in the environment for indeterminate periods and continuing infection of susceptible animals via indirect transmission. As a result, interventions that may suppress CWD prevalence in cervid populations may not prevent cycles of reinfection. Wildlife agencies carry out other management strategies that aim to slow spread, but further work is needed to evaluate the effectiveness of proposed disease interventions on local cervid population health, because outcomes are not consistently measured, if at all.
- Even with the potential development of improved disease-management measures, implementation depends on hunter acceptance and participation. Because hunters are the primary agents of cervid population management, maintaining their interest and engagement is an essential priority for wildlife agencies. However, a general decline in hunting participation, attributed to a variety of demographic changes (e.g., older hunters "aging out," lower rates of participation in younger age-groups), has been observed. As a result, wildlife agencies must balance ideal CWD management measures with approaches acceptable to hunters, which can vary for different reasons. For example, hunters who established traditions before the detection of CWD in their area may be more resistant to any regulations that could alter those familiar habits. Alternatively, those who are relatively new to the sport and are hunting in areas with the disease might view CWD as a norm and be more open to change. Regardless, without adequate levels of hunter support and participation, agencies face significant barriers, including social and political resistance and insufficient funding for the sustained implementation of disease-control methods such as targeted lethal removals (i.e., sharpshooting), which can hinder management efforts. Notably, agencies rarely aim to engage non-consumptive wildlife users, such as conservation groups and members of the general public, whose involvement could provide opportunities to garner additional support for CWD management activities, because these groups almost never contribute economically to agency budgets.

CWD Surveillance in Wild Cervids

With documented CWD detections among wild cervid populations in at least 35 states and four Canadian provinces, a growing number of wildlife professionals recognize that CWD eradication is impractical with current approaches (USGS 2024). As a result, most wildlife agencies promote early detection through surveillance of hunter-harvested and road-killed animals and implement strategies to help prevent introduction of the disease or limit its spread after detection in a new area. While modeling tools are available to predict where CWD-positive animals are most likely to be found (Ahmed 2024), many wildlife agencies take a reactive approach, waiting for initial detections before implementing

systematic surveillance or biosecurity measures.

In some states (or portions of states with high CWD prevalence), sample submission is mandatory. In other areas, sample submission is voluntary, with a number of different approaches, and costs are either subsidized by the agency or paid for by the hunter submitting the sample. A number of states, such as Minnesota, employ a risk-based approach to surveillance based on detected disease prevalence (Figure 2). Although state-run CWD testing programs are available in most states, access to testing varies considerably. Despite the perceived advantages of CWD prevention over disease suppression, implementing preventive activities is challenging, owing to sociocultural (e.g., hunter attitudes), economic (i.e., most agencies are financially ill-equipped to manage CWD), and political considerations (Delahay 2009).

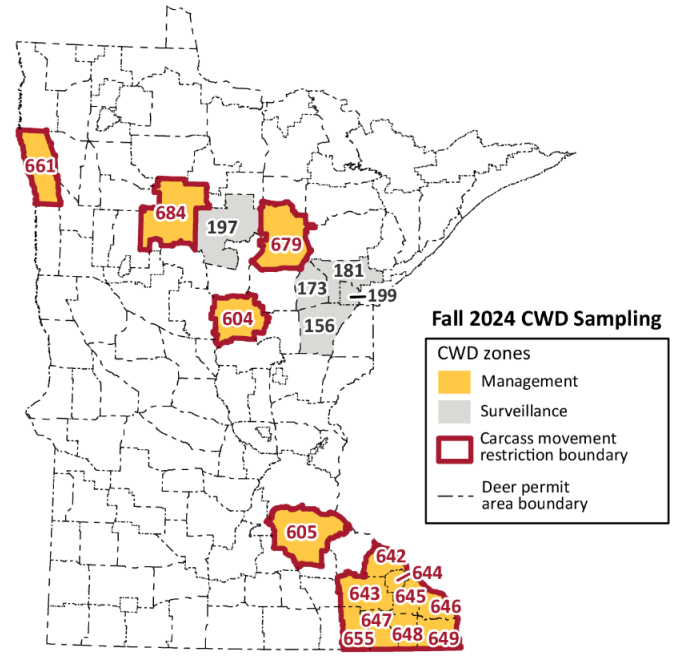


Figure 2. Chronic Wasting Disease Sampling in Minnesota (Source: Minnesota DNR 2024a)

CWD strain data and strain distribution in wild cervids are rarely collected as part of surveillance efforts, and an overview of strain-specific prevalence across North America is not currently available. This shortfall is one of several gaps in surveillance that limit the overall understanding of CWD risk, because strain variability may influence spread and the potential for spillover.

CWD Testing of Wild Cervids and Challenges

The typical workflow begins with submission of lymphatic or brain tissue (or a deer head from which the agency collects lymphatic or brain tissue) to the wildlife agency before samples are forwarded to an accredited laboratory, which must be part of the National Animal Health Laboratory Network (NAHLN) and approved by the US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) for CWD testing (Figure 3). While all US-accredited CWD testing laboratories use either enzyme-linked immunosorbent assay (ELISA) or immunohistochemistry (IHC), not all labs are approved for or use both testing methods. Because the ELISA platform offers higher-throughput testing at a lower cost than IHC, nearly all laboratories use ELISA as a screening test for hunter-harvested samples and conduct confirmatory testing with IHC. The overall sensitivity and specificity of detection methods depend on factors such as cervid species, genetics, sample quality, tissue type, and disease stage (Rivera 2019).

In recent years, sampling kits that use seed amplification assays (SAAs) such as real-time quaking-induced conversion (RT-QuIC) have become commercially available. These tests represent relatively new technology that the USDA is evaluating but has not yet approved for use in farmed cervids. Several state wildlife agencies have expressed interest in using this new technology, although most are reluctant to move forward prior to USDA approvals.

Efforts to improve CWD surveillance in free-ranging cervids have important limitations:

- Laboratory capacity is often insufficient to expeditiously meet the massive influx of samples submitted during hunting seasons in the autumn and winter. The

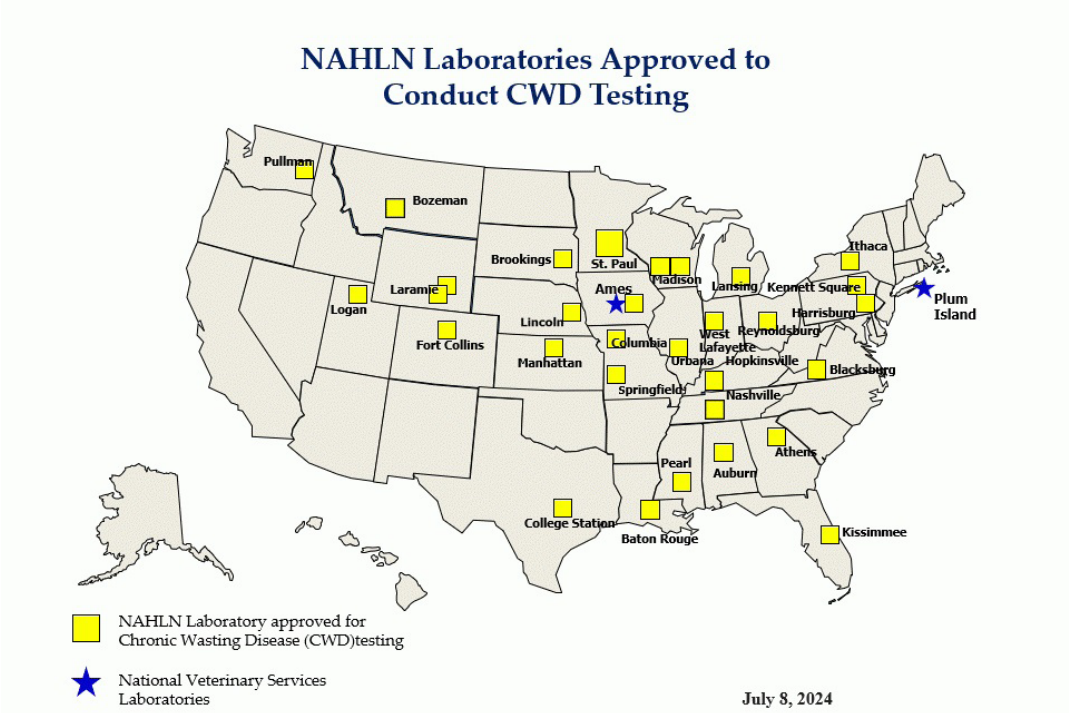


Figure 3: NAHLN Laboratories Approved to Conduct CWD Testing (Source: USDA 2024b)

large volume can result in bottlenecks that lead to prolonged wait times for CWD test results, an inconvenience and disincentive for hunters. Because testing costs nearly always require the reallocation of wildlife agency resources, sustainable surveillance and testing approaches are difficult to achieve and maintain. While NAHLN laboratories can forward samples to other network labs—which may expedite results—they are not incentivized to do so, because completing these tests is part of their operating revenue stream.

- Issues around expanding diagnostic capacity are complex, and establishing additional prion testing laboratories may actually inversely impact testing optimization. For example, because participation in NAHLN testing is voluntary, scaling up testing is at the discretion of the involved state and diagnostic laboratories. Beyond the substantial associated costs, capacity-building is often a slow process requiring forethought and patience from stakeholders. This type of facility requires considerable resources to build, staff, and maintain—all of which are problematic because most cervid sample submissions are seasonal. Furthermore, because currently available CWD tests are not certified food-safety tests in North America, state agencies are unwilling to allocate funding for a disease not yet demonstrated to threaten human and public health.

- Owing to a complex array of ecological and sociopolitical differences—such as resource availability, cervid population size and structure, and hunter participation—CWD testing programs across states, provinces, territories, and tribal nations are highly variable, with sampling quotas and sampling areas constrained by factors other than CWD. As a result, disease prevalence and distribution estimates across jurisdictions are rarely comparable. Moreover, inconsistencies in surveillance methods and funding may create gaps that allow unmonitored disease spread in cervids, increasing the risk of exposure to CWD

prions for other species, including humans.

Wild Cervid Population Management

The most common and publicly acceptable method of suppressing growing CWD prevalence and spread is hunting, with recent estimates of 6.18 million deer harvested annually in the United States (Kip Adams, personal communication). Through the implementation of various regulations, wildlife agencies can leverage hunting as a CWD management tool. Although the scope and scale of these regulations are context-dependent and often differ between jurisdictions, examples include increased harvest quotas and new or extended hunting seasons (Thompson 2023). By participating in such efforts, hunters actively assist in managing the disease. In addition, when hunters submit tissue samples from their harvested cervids for testing, they also contribute to ongoing surveillance efforts. In areas where harvest testing is not required, the willingness of hunters to participate in surveillance efforts has declined, in part due to associated costs and opposing motivations, creating additional barriers to disease management. Previous studies have demonstrated that a large portion (27% to 49%) of surveyed hunters in Tennessee, North Carolina, and South Carolina were willing to pay for CWD testing, and willingness to pay was positively associated with CWD risk perception and trust in the wildlife agency (Adhikari 2023, Lerose 2024).

Local wild cervid populations with CWD also may be managed by state-funded, targeted removals in which sharpshooters kill deer, typically outside of hunting and fawning seasons. While this method can be effective in slowing the spread of CWD, it is typically cost-prohibitive and controversial, with opposition from many hunters and landowners (whose permission is required for culling on private land). A nationwide survey in Canada focused on CWD management found negative attitudes toward the use of sharpshooting on public and private lands among the general public, hunters, landowners, and rural residents who responded (Durocher 2022). In contrast, an Illinois survey that compared hunter attitudes from 2012 and 2022 found that the practice was more accepted in the latter year, perhaps because respondents saw the benefits of this strategy (Vaske 2024).

Finally, various predator-advocate organizations have proposed wolf, bear, and mountain lion introductions to suppress CWD. In part, this approach assumes that predators preferentially remove older or sick animals. However, only limited empirical evidence suggests that predator introductions suppress CWD at the population level (Chance 2022, Miller 2008). More broadly, predator population dynamics often have unpredictable effects on the ecosystem that must be carefully considered before implementing this often-controversial approach. Moreover, predators may actually facilitate CWD spread by scattering prey animal remains or distributing prions in their feces following consumption (Nichols 2015), although the true risk of this is not yet fully understood. For example, results from one study that involved feeding CWD-infected material to mountain lions indicated that most of the ingested prions were eliminated or otherwise sequestered during digestion, which may ultimately dilute the infectivity of prions passed through the gastrointestinal tract of this predator (Baune 2021). Although some research suggests that certain predator species appear resistant to prion infection after repeated exposure to prions (Wolfe 2022), the presence of an intermediate host or cannibalism could facilitate adaptation of the agent and lead to disease (Barrio 2024). For example, feline spongiform encephalopathy (FSE), a prion disease of domestic cats and captive wildcats, has been linked to consumption of BSE-contaminated meat (Imran 2011). CWD has been experimentally transmitted to domestic cats inoculated with prions intracerebrally on a first passage or orally (with tongue abrasions) on a second passage (Mathiason 2013).

CWD Surveillance and Response in Captive Cervid Operations

CWD also impacts captive cervids raised behind high-fence enclosures. Although US state approaches to CWD detection and control among captive cervids, wildlife and agricultural agencies vary significantly, they typically share regulatory jurisdiction and cooperate with USDA's APHIS Veterinary Services program. APHIS and state agencies also cooperate to certify facilities in their jurisdictions under the USDA CWD Herd Certification Program (HCP). The Canadian Food Inspection Agency (CFIA) operates a similar program known as the CWD Herd Certification Program. As of December 2024, 28 US states were participating in the USDA's CWD HCP, and six Canadian provinces were participating in the Canadian program.

The goal of the US CWD HCP is to provide a consistent national approach to control the incidence of CWD in farmed cervids and prevent its interstate spread (USDA 2024a). To be certified by the USDA as low risk for CWD, enrolled herd owners must reach 5 years of program compliance with national CWD HCP requirements. This includes fencing, official individual animal identification, detailed record keeping, and CWD testing of all deaths of cervids 12 months or older by immunohistochemistry (IHC) of both the retropharyngeal lymph nodes and the obex region of the brain. The APHIS National Veterinary Services Laboratory (NVSL) in Ames, Iowa, performs all confirmatory testing for CWD in farmed cervids. As with detections in wild cervids, when CWD is detected in farmed animals, the agencies craft messaging and announce the detection to the public. To meet minimum regulatory requirements of the national program (states may have additional or stricter requirements), enrolled herds with CWD-positive animals immediately lose HCP herd status, and the producer must quarantine the herd. The herd may re-enroll in the HCP after entering into a herd plan, with options for responding to a CWD-positive herd, including complete depopulation and postmortem CWD testing, quarantine for 5 years after the last CWD-positive case, and antemortem CWD testing and genotyping using NVSL protocols and APHIS-approved procedures (USDA 2019).

The USDA has approved three postmortem diagnostic methods to identify CWD prions: ELISA, IHC, and Western blot (WB), although WB is an official test only when performed at NVSL and is not used as a diagnostic assay for general surveillance. Antemortem IHC testing can also be used in captive cervids when specific criteria are met (e.g., trace-out herds, producers with positive herds wishing to reduce the presence of CWD-positive deer, outlined in herd plan, herd exclusively contains white-tailed deer, codon 96 genotypes for the white-tailed deer have been established). The USDA has yet to validate amplification assays such as protein misfolding cyclic amplification (PMCA) and RT-QuIC for diagnosis of CWD.

Through program-required surveillance and biosecurity measures, CWD HCP regulations are intended to detect the presence of CWD as soon as possible and prevent positive animals from moving out of captive herds. However, HCP participation is voluntary. Detections of CWD across the United States and Canada continue to occur among captive herds, even in some certified by the HCP (Gillin 2018). Similar to wild cervid herds, some captive cervid facilities (not all enrolled in HCP) have reported CWD herd infection rates of 70% or higher. CWD in farmed animals can also affect nearby wild populations, because transmission can occur if wild animals gain access to or make contact through the facility's fence (double-fencing is not required in all jurisdictions) or come into contact with escaped animals when fencing is breached.

The risk of CWD spread continues beyond the point of depopulation because prions can persist in the environment for long periods, on the order of years. Often, states require that owners maintain the fence surrounding an inactivated, CWD-positive cervid facility, but it

is unclear what length of time is sufficient to allow prion inactivation and elimination of risk to wild populations.

Other Wildlife Regulatory Measures

Beyond population reduction, wildlife agencies may also implement primary prevention–based regulations aimed at keeping CWD contamination from reaching susceptible cervid populations. Examples of such regulations include feeding bans, carcass transport restrictions, and bait/attractant restrictions or bans. Artificially attracting wild cervids to a location promotes unnatural congregations of wild animals, increasing opportunities for disease transmission. Because every state or province with CWD may enact its own disease-control regulations, practices vary by location. For example, some CWD-positive jurisdictions, such as South Dakota, explicitly ban baiting for hunting purposes while allowing supplemental feeding outside of hunting season; states such as Georgia have conditional bans that depend on whether the activities are taking place in or around CWD management zones (CWD Alliance 2021). In Minnesota, the Department of Natural Resources (DNR) restricts deer feeding and the use of attractants in certain counties (Figure 4). CWD regulations are subject to change based on government administrations and measured disease prevalence, which may influence public adherence. The CWD Alliance published its interactive "CWD Hunting Regulations Map," with more specific state, provincial, and territorial information, in 2021 (CWD Alliance 2021).

Restricting the transportation of carcasses aims to reduce opportunities for infected materials to contaminate the environment with prions. Because prions can remain infectious in the environment for years or decades, the logical inference is that the more an infected animal moves, the wider the dispersal of prions via direct contact and environmental contamination. Nevertheless, currently, agencies have no way to empirically measure the effectiveness of these tactics.

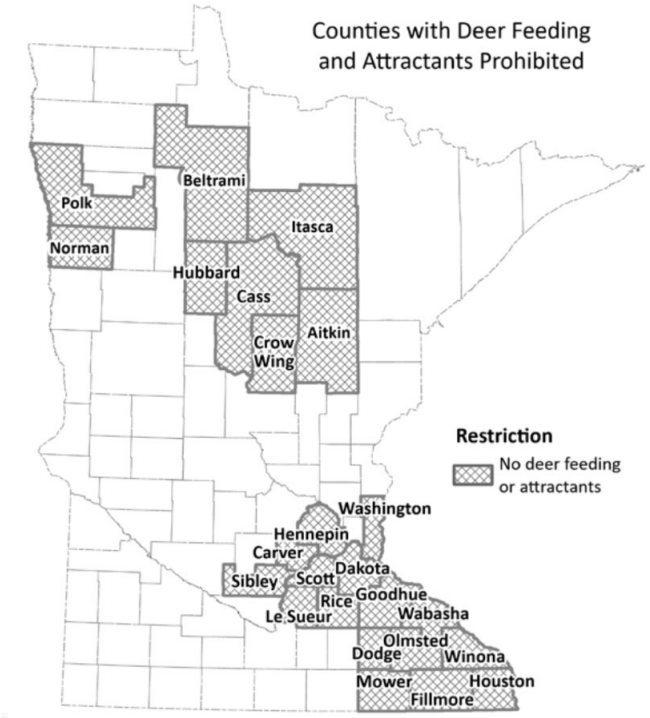


Figure 4: Minnesota Counties with Deer Feeding and Attractants Prohibited (Source: Minnesota DNR)

CWD Management in Tribal Nations

Hunting practices among Native American tribes vary greatly across North America, but tribal populations frequently rely on cervid hunting for sustenance and cultural traditions (Parlee 2021). Hunting is a significant tradition deeply rooted in the intergenerational celebration of the relationship between Native people, the animals that provide nourishment, and the spiritual connection to the environment. Thus, tribal governments are key stakeholders in CWD management, although they have been and continue to be underrepresented in disease control and management conversations. The US government holds treaties with tribal nations, but state governments do not (Schwabenlander 2022). Further, because state governments have no authority over tribal lands or hunting practices, coordinating CWD management efforts in these settings requires establishing partnerships with tribal natural resource agencies. The structure and strength of these relationships vary but often depend on reservation size and configuration,

local CWD prevalence, legal or treaty relationships, and available resources.

Tribes often issue their own hunting licenses, and these can differ from non-tribal licenses in terms of season dates, who can use the license to harvest an animal, and harvest quotas. Although practices vary by band or tribe, licenses to hunt on tribal lands can be granted to band members, non-band members, and non-Indian hunters (Mille Lacs Band of Ojibwe). However, in states and provinces where tribes have usufructuary rights (i.e., the right to use the land) but limited tribal-owned land, tribal members may elect to hunt on land outside their reservation. These rights cross state borders; for example, treaty rights allow tribal members in the Upper Peninsula of Michigan to hunt in portions of neighboring Wisconsin and Minnesota and vice versa.

Regardless of where an animal is harvested, tribal members in North America who have their deer tested for CWD generally rely on state and provincial departments of natural resources for this service (Parlee 2021). Results from Minnesota tribal hunters are reported to the tribal natural resource agency, which conducts its own surveillance and can voluntarily share it with the state DNR (Minnesota DNR 2024b).

In tribal communities, a spillover event into either livestock or humans would be greatly disruptive because of the importance of cervids to tribal traditions, culture, and in some instances, a source of protein. Beyond potential risk associated with the consumption of CWD-positive meat, tribal members may engage in practices such as the brain-tanning of hides that may further increase the risk of spillover events. As a result, certain aspects of CWD management in tribal communities differ from those experienced elsewhere and warrant additional consideration.

Public Education and Information Sharing

CWD testing information and risk-communication messaging is available in most jurisdictions, but the delivery, consistency, and timeliness of such information varies. Typically, state or provincial agencies overseeing the regulation of cervid populations and CWD management communicate new detections, usually followed by a press release. The US Geological Survey (USGS) map (Figure 1) captures and presents information to the public about detections in North America each month.

CWD public messaging regarding exposure primarily targets hunters. Engagement with educational materials typically relies on timely, accurate messaging and can be influenced by resistance to governmental guidance, participation fatigue, and unwillingness to change hunting behaviors. Furthermore, after CWD has been present in an area for a time, hunters almost invariably begin to disregard or ignore outreach materials. A survey conducted in Wisconsin, for example, asked subjects what they did with harvested venison after learning their animal was CWD-positive. Of those surveyed, 27% reportedly ate the venison, 9% said other family members ate it, and 10% gave it to friends (Bradshaw 2021). Given the continually evolving nature of CWD (e.g., strain emergence, spread to new areas, higher prevalence, more exposure), providing up-to-date communications at the local level is critical to address declining adherence to CWD guidelines and re-energize compliance with regulations.

Ongoing and Future Implications Associated with Growing CWD Prevalence

Potential Spillover to Non-Cervid Wildlife

Among the factors not addressed by current CWD surveillance efforts is potential transmission to a sympatric (sharing the same habitat) wild species. While researchers are

unclear as to which non-cervid species are at greatest risk for prion infection, several experimental interspecies transmission studies have demonstrated prion susceptibility to other hosts, although many of these studies have involved intracerebral inoculation (Heisey 2010, Kurt 2016). Bank voles are a common model species for studying CWD because they commonly share a natural habitat with cervids, and certain genotypes are highly susceptible to CWD (Nonno 2020). Leveraging this knowledge, additional research has demonstrated that raccoons have limited susceptibility to CWD prion isolates from white-tailed deer and elk (Moore 2022). Current research is also investigating transmission potential to feral swine, because experiments in porcine models have shown moderate levels of CWD amplification (Moore 2017).

Findings that CWD can infect non-cervid species may change risk perceptions for other wildlife, but the extent of these implications is unclear because, without surveillance, no information is available to determine spread among free-ranging populations. Expanded surveillance, if feasible, would be difficult to implement owing to challenges in identifying the populations to sample, the diagnostic tests to use, and available funding sources. Furthermore, as federal regulations are currently written, CWD is not reportable in species other than farmed cervids, which complicates the ability to conduct expanded surveillance.

Impacts on Hunting

Sales of deer hunting licenses, the most important source of revenue for state fish and wildlife agencies, are essential for maintaining North American wildlife conservation (AFWA). Among other threats, CWD reduces license purchases, influences harvest, and creates animosity between stakeholders and government agencies. For example, hunter surveys in Colorado suggest that if disease prevalence increased from 10% to 50%, 30% of respondents reported that they would stop buying licenses and hunting in Colorado (Quartuch 2024). Multistate surveys from 2004 and 2006 reported similar results: 49% of respondents reported that they would stop hunting altogether if most cervids in their area were infected with CWD (Harper 2015).

Impacts on Herd Composition

Reducing the incidence of CWD among wild cervids may diminish prevalence but will not eliminate the disease. Although data from southeastern Wyoming, northeastern Colorado, and southwestern Wisconsin are concerning, much remains unknown about the long-term impact of CWD on wild cervid populations and the external environmental factors that could contribute to population dynamics in the presence of CWD (DeVivo 2017, Edmunds 2016). In some areas, data suggest that CWD may wipe out local populations, although in other settings, CWD may impact sex and age ratios but not affect overall abundance (Haworth 2021, Rogers 2022). Given the myriad of variables that could influence long-term outcomes of CWD on cervid herds (e.g., cervid species, population density, habitat), it is difficult, if not impossible, to comprehensively forecast the impact CWD might have on cervid populations and the associated ramifications for ecosystems. Regardless of impact size and timing, the persistence of CWD in wild cervid populations will result in some level of negative outcomes for affected herds (Uehlinger 2016).

Key Findings

- Because the management of wild cervid populations generally falls under the jurisdiction of state and provincial governments, approaches to CWD surveillance and management across North America vary significantly both within and between jurisdictions, including tribal jurisdictions, owing to differences in available resources and political willingness to address the disease.
- Wildlife agencies rely on voluntary hunter harvest as the most important mechanism for CWD disease surveillance and management. As a result, agencies are challenged to maintain hunter engagement on this issue. This can be complicated, given the long-term nature of the disease and the perceived burdens that may accompany certain actions, both of which contribute to hunter "disease fatigue."
- At the time of the initial publication of this report, no effective methods are available to eliminate CWD in wild cervid populations.
- Deer, elk, moose, and other cervids are keystone species ecologically and economically, and they are a vital source of protein and a cultural cornerstone for groups such as tribal communities and rural populations. CWD may pose an existential threat to these species and generational hunting traditions.
- Wildlife agency budgets already face constraints because of declining hunting license sales (primarily for deer hunting) and the rising costs of CWD management and surveillance. Documented CWD spillover into humans or non-cervid production animals will exacerbate these funding challenges, underscoring the need for additional support and contingency plans that consider such events.
- While different prion strains can have important implications for disease dynamics, the diversity of CWD strains on the cervid landscape is virtually unknown. Most CWD surveillance programs do not collect strain-level data.
- Surges in demand during annual hunting seasons challenge existing laboratory capacity for CWD testing. Without additional capacity or the availability of less expensive, high-throughput tests, any surge in demand prompted by CWD spillover will likely overwhelm available testing systems.
- The structures and functions of wildlife agencies are not conducive to proactively addressing big-picture challenges such as potential CWD spillover. Moreover, even with the current reactive approach, maintaining a stable response to a "bad-news issue" such as CWD is complicated by fluctuating political landscapes, changes in agency leadership, waning public interest, and limited resources.
- Because CWD surveillance is not conducted in non-cervid sympatric wild species, the potential for CWD spillover to other wildlife, particularly small mammals, is unknown.
- Despite the potential One Health implications of CWD, wildlife agencies that oversee its management in free-ranging cervids understandably approach the disease exclusively as a wildlife health concern, and the involvement of other agencies such as those involved with public health has been limited. However, if documented CWD spillover occurs, the role of wildlife agencies would change significantly.
- Federal officials have a greater role in managing CWD in farmed cervids through herd certification programs in the United States and Canada. However, even with

such programs in place, CWD continues to spread among farmed cervids in both countries, posing an unquantified but likely growing risk to free-ranging animals.

References

Adhikari RK, Poudyal NC, Muller LI, et al. Hunter willingness-to-pay for disease testing: Evidence from chronic wasting disease in white-tailed deer. JAAE. 2023 Aug 7;55(3):417-31 [Full text]

Association of Fish and Wildlife Agencies. Hunting & Fishing License Sales and the Wildlife & Sport Restoration Trust Funds [Web page]

Ahmed MS, Hanley BJ, Mitchell CI, et al. Predicting chronic wasting disease in white-tailed deer at the county scale using machine learning. Sci Rep. 2024 Jun 22;14(1):14373 [Full text]

Barrio T, Benestad SL, Douet JY, et al. Zoonotic potential of chronic wasting disease after adaptation in intermediate species. Emerg Infect Dis. 2024 Dec;30(12):2691-4 [Full text]

Baune C, Wolfe LL, Schott KC, et al. Reduction of chronic wasting disease prion seeding activity following digestion by mountain lions. mSphere. 2021 Dec 22;6(6):e0081221 [Full text]

Bradshaw L, Pluemer M, Beardmore B, et al. Deer Hunters' Attitudes and Behaviors Related to Chronic Wasting Disease (CWD) in Wisconsin in 2019. Wisconsin Department of Natural Resources. 2021 Dec [Full text]

Chance A, Newsome C, Brandell E, et al. Predator impacts on the spread of chronic wasting disease. National Wildlife Federation Blog. 2022 Oct 20 [Full text]

Chiavacci SJ. The economic costs of chronic wasting disease in the United States. PLoS One. 2022 Dec 8;17(12):e0278366 [Full text]

Chronic Wasting Disease Alliance. CWD Hunting Regulations Map. 2021 [Web page]

CWD Research Consortium. Participants. [Web page]

Delahay RJ, Smith GC, Hutchings MR. The science of wildlife disease management. In: Management of Disease in Wild Mammals. 2009 [Full document]

DeVivo MT, Edmunds DR, Kauffman MJ, et al. Endemic chronic wasting disease causes mule deer population decline in Wyoming. PLoS One. 2017 Oct 19;12(10):e0186512 [Full text]

Durocher G, Luckert MK, Goddard EW, et al. Public preferences for options to manage chronic wasting disease in Canada: A paired comparisons study. Environ Sci Policy. 2022 Mar 8;132:206-13 [Full text]

Edmunds DR, Kauffman MJ, Schumaker BA, et al. Chronic wasting disease drives population decline of white-tailed deer. PLoS One. 2016 Aug 30;11(8):e0161127 [Full text]

Gillin C, Mawdsley J, eds. AFWA Technical Report on Best Management Practices for Prevention, Surveillance, and Management of Chronic Wasting Disease. Association of Fish and Wildlife Agencies. 2018 [Full document]

Harper EE, Miller CA, Vaske JJ. Hunter perceptions of risk, social trust, and management of chronic wasting disease in Illinois. Hum Dimens Wildl. 2015 Sep 3;20(5):394-407 [Full text]

Haworth SE, Nituch L, Northrup JM, et al. Characterizing the demographic history and prion protein variation to infer susceptibility to chronic wasting disease in a naïve population of white-tailed deer (*Odocoileus virginianus*). Evol Appl. 2021 Mar 30;14(6):1528-39 [Full text]

Heisey DM, Mickelsen NA, Schneider JR, et al. Chronic wasting disease (CWD) susceptibility of several North American rodents that are sympatric with cervid CWD epidemics. J Virol. 2010 Jan;84(1):210-5 [Full text]

Imran M, Mahmood S. An overview of animal prion diseases. Virol J. 2011 Nov 1;8:493 [Full text]

Kurt TD, Sigurdson CJ. Cross-species transmission of CWD prions. Prion. 2016;10(1):83-91 [Full text]

Lerose CS, Peterson MN, Levine JF, et al. What are hunters willing to pay for chronic wasting disease (CWD) management? A comparison of different contingent valuation approaches. Southeastern Association of Fish and Wildlife Agencies. 2024;11:67-75 [Full text]

Mathiason CK, Nalls AV, Seelig DM, et al. Susceptibility of domestic cats to chronic wasting disease. J Virol. 2013 Feb;87(4):1947-56 [Full text]

Mille Lacs Band of Ojibwe. Subchapter 1 - General Provisions [Web page]

Miller MW, Swanson HM, Wolfe LL, et al. Lions and prions and deer demise. PLoS one. 2008 Dec 24;3(12):e4019 [Full text]

Minnesota Department of Natural Resources. Chronic wasting disease management. 2024 [Web page]

Minnesota Department of Natural Resources. CWD test results: July 1, 2024 to present. 2024 [Web page]

Minnesota Department of Natural Resources. Deer feeding and attractants bans. [Web page]

Minnesota Legislature. Minnesota Statutes 2023 97A.405 License Requirements. 2023 [Full document]

Moore SJ, West Greenlee MH, Kondru N, et al. Experimental transmission of the chronic wasting disease agent to swine after oral or intracranial inoculation. J Virol. 2017 Oct 1;91(19):e00926-17 [Full text]

Moore SJ, Carlson CM, Schneider JR, et al. Increased attack rates and decreased incubation periods in raccoons with chronic wasting disease passaged through meadow voles. Emerg Infect Dis. 2022 Apr;28(4):793-801 [Full text]

Nichols TA, Fischer JW, Spraker TR, et al. CWD prions remain infectious after passage through the digestive system of coyotes (*Canis latrans*). Prion. 2015 Sep-Oct;9(5):367-75 [Full text]

Nonno R, Di Bari MA, Pirisinu L, et al. Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America. Proc Natl Acad Sci USA. 2020 Dec 8;117(49):31417-26 [Full text]

Parlee B, Ahkimnachie K, Cunningham H, et al. "It's important to know about this"—Risk communication and the impacts of chronic wasting disease on indigenous food systems in Western Canada. Environ Sci Policy. 2021 Sept;123:190-201 [Full text]

Quartuch MR, Studebaker S. Differences between resident and nonresident hunters' perceptions about CWD and associated implications: A case study from Colorado. Hum Dimens Wildl. 2024 Aug 6;1-14 [Full text]

Rivera NA, Brandt AL, Novakofski JE, et al. Chronic wasting disease in cervids: Prevalence, impact and management strategies. Vet Med (Auckl). 2019 Oct 2;10:123-39 [Full text]

Rogers W, Brandell EE, Cross PC. Epidemiological differences between sexes affect management efficacy in simulated chronic wasting disease systems. J Appl Ecol. 2022 Apr;59(4):1122-33 [Full text]

Schwabenlander MD, Potts N, Moore S, et al. Upper Midwest tribal natural resource managers' perspectives on chronic wasting disease outreach, surveillance, and management. Conserv Sci Pract. 2022;4e12710 [Full text]

Thompson NE, Huang MHJ, Christensen SA, et al. Wildlife agency responses to chronic wasting disease in free-ranging cervids. Wildl Soc Bull. 2023 Mar 28;47(2):e1435 [Full text]

Thompson NE, Mason JR. The cost of combatting chronic wasting disease: Putting a dollar amount on annual CWD expenses by state wildlife agencies. Association of Fish and Wildlife Agencies. 2022 [Full document]

Uehlinger FD, Johnston AC, Bollinger TK, et al. Systematic review of management strategies to control chronic wasting disease in wild deer populations in North America. BMC Vet Res. 2016 Aug 22;12(1):173 [Full text]

US Department of Agriculture Animal and Plant Health Inspection Service. Cervids: CWD Voluntary Herd Certification Program. 2024 Sep 6 [Web page]

US Department of Agriculture Animal and Plant Health Inspection Service. Chronic Wasting Disease (CWD) Laboratories. 2024 Sep 25 [Web page]

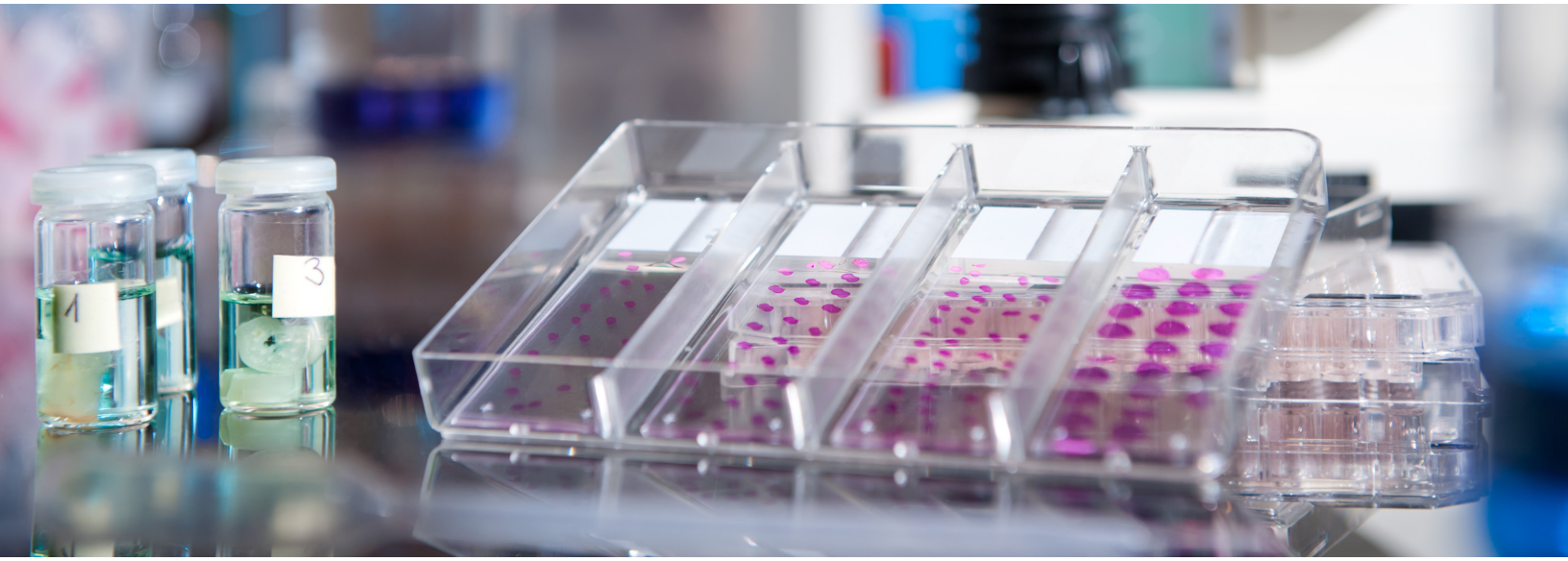
US Department of Agriculture Animal and Plant Health Inspection Service. Chronic Wasting Disease Program Standards. 2019 May [Full document]

US Fish and Wildlife Service. 2016 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation. Revised 2018 Oct [Full document]

US Geological Survey. Distribution of Chronic Wasting Disease in North America. 2024 Dec [Web page]

Vaske JJ, Miller CA. Predicting hunter beliefs about sharpshooting in controlling CWD in Illinois over time. Hum Dimens Wildl. 2024 Jan 5 [Full text]

Wolfe LL, Fox KA, Griffin KA, et al. Mountain lions (*Puma concolor*) resist long-term dietary exposure to chronic wasting disease. J Wildl Dis. 2022 Jan 1;58(1):40-9 [Full text]



Chapter 2: CWD Diagnostic Laboratory Testing in Animals

Introduction

Laboratory diagnostic testing is the cornerstone of understanding the epidemiology of any disease, including CWD. Tests currently approved by the USDA and CFIA for the detection of CWD in cervids include IHC, ELISA, and WB. Each of these tests has advantages and limitations, and all methods require substantial investments in personnel, training, and equipment. The limitations of current testing have led to the development of next-generation tests, including prion-seeded amplification assays. Although these assays are used for the diagnosis of human prion disease, none of these next-generation tests have been approved by the USDA for use with cervids. As validation testing proceeds, these newer and more sensitive assays have advanced CWD research (McNulty 2019). This chapter reviews currently validated diagnostic testing for CWD in cervids, summarizes the status of next-generation testing, identifies important challenges in CWD testing in cervids and non-cervid species, and outlines key issues in identifying CWD in production animals, if a spillover from a wild cervid to a production animal should occur.

Approved CWD Diagnostic Testing in Cervids

CWD Screening Testing

In most instances, tissue samples from farmed and hunted cervids tested for CWD are screened with ELISA first, because this test is relatively easy to perform, inexpensive, high-throughput, and has a relatively short turnaround time (USDA 2019). An early field study demonstrated a sensitivity and specificity of greater than 97% for ELISA testing of samples taken from RPLN, with IHC testing as the comparator (Hibler 2003); however, the performance profile of ELISA testing across the complete spectrum of variable field conditions (e.g., cervid species, *Prnp* genotype, tissue type, stage of infection) is underexplored. ELISA testing for CWD uses a targeted, labeled antibody to signal the concentration of prion proteins in a sample (Burgener 2022). Validated ELISA test kits used for the surveillance of several prion diseases (i.e., CWD, BSE, and scrapie) in

Canada and the United States are available from only one source (Bio-Rad Laboratories, Inc.) and are manufactured in Europe. Supply chain disruptions have resulted in ELISA test kit shortages, which have caused backlogs in CWD testing (Wappes 2023). Another manufacturer, IDEXX, produces ELISA test kits demonstrated to be more sensitive than the Bio-Rad alternative, but validation studies would need to be conducted in the United States and Canada to include these test kits as an option in standard operating procedures (Mazza 2023). Nonetheless, owing to its high throughput and ability to detect CWD, BSE, and scrapie prions, ELISA testing, when readily available, can increase workflow efficiencies. As a result of its versatility, ELISA testing may also have some capacity to identify CWD prions in samples from non-cervid hosts, although additional research is needed to explore this possibility. Of importance, ELISA screening cannot differentiate between CWD prion strains.

CWD Confirmatory Testing

In captive cervid testing programs, all samples that test "non-negative" by ELISA must be sent for confirmatory testing. A positive ELISA (or IHC) test result from any USDA HCP sample submitted to an approved laboratory is considered a CWD-suspect test result until confirmed by IHC at NVSL. In Canada, ELISA-positive samples are sent to the CFIA Reference Laboratory for CWD for confirmatory testing. Confirmatory testing in captive cervids is especially important because of the regulatory and economic implications of CWD in this setting (e.g., the potential for depopulation, other actions that impact the herd). Confirmatory testing is generally not performed on samples from ELISA-positive wild cervids originating in disease-endemic areas. However, confirmatory testing is often advised when CWD is detected in a new area, new herd unit, or new species.

For more definitive CWD diagnosis, the regulatory laboratories designated by their respective agencies can use two confirmatory tests: IHC and WB. IHC is a thoroughly validated and reputable testing method that allows visualization of disease-associated prion inclusions in samples by directing labeled antibodies toward an epitope of the prion protein. This method exhibits high sensitivity and specificity for diagnosing clinical prion disease from RPLN and obex brain tissue (Schneider 2023). Depending on samples available for analysis, IHC can also help characterize the localization of prion deposits in tissues outside the lymphatic and central nervous systems (Seelig 2011). With its typical application as a postmortem, confirmatory test, IHC can detect and visualize disease-associated prion protein deposits in cervid tissue postmortem.

WB not only detects CWD prions but also can be used to explore prion biochemical properties such as resistance to proteinase K activity, which discriminates normal host prion proteins, or PrP^{C}, from PrP^{Sc}, the detection of which is pathognomonic of prion infection (Figgie 2021, Sajnani 2012). As a result, the NVSL and CFIA's reference laboratory uses WB testing by to provide additional evidence of infection following inconclusive IHC results or as a confirmatory test if the tissue samples are frozen. In some circumstances, WB can also help distinguish prion types (e.g., CWD vs BSE), and distinctions in WB banding patterns have also been observed between CWD strains (Otero 2022, CFIA 2020, Nonno 2020). It is important to note, however, that for some prion types or strains, researchers have not identified any apparent differences in WB results (i.e., banding patterns are indistinguishable).

Animal Bioassays

Animal bioassays, which involve inoculation of samples from infected animals to natural or transgenic hosts, are the definitive method for assessing prion infectivity (McNulty 2019). Early longitudinal studies involving exposure of uninfected natural hosts to material from infected animals confirmed the transmissibility of CWD prions in cervid populations and also uncovered potential transmission routes (Mathiason 2006, McNulty 2019). Transgenic

mouse models, which are genetically altered to express the PrP^C of select species (typically cervids for bioassays involving CWD), have been developed to more thoroughly explore issues related to CWD transmission, including investigating routes of transmission, the potential for environmental transmission, cross-species transmission and species barriers, and the effects of strain variability on transmission (Browning 2004, Cook 2023). More recently, gene-targeted mice (i.e., so-called "knock-in" mice) have been developed that may more accurately depict transmission potential and pathology (Sun 2023). Other animal models for studying CWD have also been developed, including white-tailed deer, reindeer, nonhuman primates (NHPs), and other small animal models such as hamsters and bank voles.

Although CWD animal bioassays are impractical for routine diagnostic purposes for a host of reasons, including cost, limited throughput, prolonged incubation periods, and ethical considerations, they play a critical role in research necessary to understand CWD transmissibility (particularly to humans and non-cervid production animals) and pathogenicity (McNulty 2019). For example, animal experiments have been used to measure infectivity titers in a given sample and demonstrate CWD transmission potential to non-cervid animal models (e.g., cows, pigs, goats, sheep). Investigating CWD susceptibility in production animals is of particular interest because of the implications to agriculture if a spillover incident were detected. As in humanized animal models, production-animal models need to simulate the mechanics of a naturally occurring transmission event. For example, experimental animal models demonstrate that the disease presents differently in non-cervid species in terms of prion tissue tropisms and disease progression.

The interpretation of the results of animal models is limited. Most lines of transgenic animals overexpress naturally occurring PrP^C by design, and this likely increases susceptibility to prion infection, especially when inoculum concentrations are high (Watts 2014). Also, some transgenic mice only express PrP^C in certain tissues of the central nervous system, which is not characteristic of prion disease in cervids or humans, in which PrP^C is located throughout the body. Therefore, the methodology and limitations of bioassay results must be considered when reviewing findings from research reports.

Challenges with the Current Wildlife CWD Testing Strategy

Although the strategy of ELISA screening followed by IHC or WB confirmatory testing is currently considered the regulatory standard for wildlife CWD testing, this approach has a number of important limitations, such as limited throughput, no environmental sample functionality, potential for supply disruptions, prolonged times to obtain results, cost, and an (as yet) undetermined capacity to handle diverse specimens from non-cervid animals in the investigation of a CWD spillover incident. In the current US model, which is based mostly on hunter-submitted samples from cervids harvested during hunting season, testing capacity is continually stressed not only because of the short window of time (i.e., hunting season) in which labs receive large volumes of tissues but also because of the lack of sampling needs throughout the remainder of the year. As noted earlier in this report, hunters harvest more than 6 million deer in the United States each year. Even though the vast majority of deer are never tested for CWD, this seasonal pressure on capacity raises questions about the sustainability of the current testing model. This is of particular concern if evidence of cross-species transmission of CWD is identified, which could dramatically increase testing demand. Additional information on CWD surveillance and testing capacities of state agencies can be found in Appendix A.

Furthermore, testing results can vary depending on factors such as sample quality, tissue type, cervid species, *Prnp* genotype, stage of CWD infection, prion concentration,

and prion strain; therefore, the true diagnostic accuracy of the current approach under field conditions is unknown (Picasso-Riso 2022). Of significance, the current testing strategy may be less sensitive in the early stages of infection, when prion accumulation is low and dissemination of prions in different tissues is variable (Picasso-Riso 2022). In vitro tests used in current CWD testing protocols also do not allow inferences about quantifiable prion infectivity in a susceptible host, and they may not differentiate between CWD strains, which could be useful in surveillance activities and is important for understanding CWD epidemiology and strain-specific transmission risks. Finally, because sophisticated equipment and technical expertise are in short supply, accessibility and timeliness differ across testing locations in the United States and Canada. These barriers impact hunter participation in CWD testing initiatives and impede the dissemination of test results.

Collectively, these limitations would also challenge the diagnostic investigation of a CWD spillover to a production animal. Assuming that tissues from the animal in question are sent to the NVSL for confirmatory analysis, precedence is lacking to inform interpretations of the results in a naturally infected animal, because results are currently available only for experimentally infected animals. A tissue repository from previous and ongoing CWD research in non-cervid animals could be an important source of reference materials to inform the diagnostic investigation. This resource would be similar to the USDA's tissue archive that maintains samples from farmed cervids and the pending tissue repository for samples from wild cervids. (USDA APHIS VS, CWD Research Consortium 2021).

Prion Seeded Amplification Assays and Potential Applications in Animals

Technology

Given the limitations of the current testing strategy in free-ranging animals, next-generation tests are needed that offer high throughput at low cost; are highly sensitive and specific, particularly in the early stages of infection; and can be used for antemortem diagnosis. To address these needs, SAAs have been developed that use the agent's (PrP^{Sc}) natural ability to convert host protein (PrP^{C}) to its misfolded, aggregated forms. These tests involve adding a small amount of tissue homogenate or fluid to an excess of compatible substrate and incubating the mixture to determine whether PrP^{C} aggregates.

In most prion PMCA assays, the source of PrP^{C} is brain homogenate, and the amplified product is infectious. In RT-QuIC assays, PrP^{C} produced in bacteria is used, and the products are not infectious. For these and other reasons, PMCA more accurately recapitulates prion replication, while RT-QuIC is more practical for routine prion detection (Bartz 2024). Both PMCA and RT-QuIC are valuable tools for diagnosing CWD infection, with results comparable to or better than ELISA screening with IHC confirmatory testing (Benavente 2023, Holz 2022). RT-QuIC can be scaled up for high-throughput, test results can be obtained relatively quickly, results become positive earlier in the course of infection, and the test is less expensive and laborious to perform than the current approach to CWD diagnosis (Holz 2022). In addition, these amplification assays have the potential to be used on tissues or fluids collected antemortem. Recent studies have begun to elucidate the relationship between disease progression and prion presence in alternative tissues or fluids. Highly sensitive SAAs may return CWD-positive results from rectoanal mucosa-associated lymph tissue (RAMALT),

even when confirmatory testing of the obex is negative for CWD. IHC could also be used to assess RAMALT and other tissues, but it is less sensitive than RT-QuIC in identifying the presence of prions. Because RAMALT samples can be acquired antemortem, the possibility of using amplification assay screening is particularly appealing to the farmed cervid industry.

Validation Challenges

As new assays are developed and optimized, the reproducibility of results and the standardization of source materials remain important issues. Determining the sensitivity and specificity of an SAA is challenging, particularly in samples with low prion titers, because few tools are available to reconcile conflicting results (e.g., detection via RT-QuIC but not ELISA or IHC). Another issue is that interpretation of RT-QuIC data for diagnostic purposes has not been standardized, complicating inter-laboratory comparisons of the relationship between prion disease status and RT-QuIC results (Rowden 2023). Thus, RT-QuIC and PMCA have not yet been validated for use in the CWD diagnostic workflow by the USDA or CFIA because of reliability concerns. However, the field has recently made strides to address this issue by converging on common reagents and nearly identical protocols (Darish 2024). Given the advantages of these platforms, federally regulated diagnostic laboratories should continue to pursue validation and verification of prion amplification assays for routine use.

Many state and provincial wildlife agencies will likely wait to implement surveillance with SAAs until they secure validation from federal regulatory agencies. In the absence of such validation, the farmed cervid industry is continuing to use the standard approach of ELISA screening followed by confirmatory testing in Canada and IHC followed by confirmatory testing in the United States. Although many state agencies would prefer to use a highly sensitive amplification assay for testing hunter-harvested cervid samples, wildlife professionals have expressed a desire to continue current procedures that align with treatment of farmed cervid samples.

Gaps in Characterizing CWD Strains

While different strains of CWD exist, and strain evolution and diversification may have important implications for CWD prion transmissibility over time, limited methodologies are available for accurate and accessible strain typing, particularly as part of CWD surveillance activities. In addition, as more testing methods become available to assess CWD strains, researchers will likely identify new strains and may refine how strains are classified. Neither ELISA nor IHC testing alone provide any CWD strain-specific information. ELISA followed by IHC characterizes the tissue tropism and histopathological profile, which can help define prion strains, but overlap in strain identity decreases distinction capabilities, and although WB can help distinguish certain CWD strains, the overall resolution is low. Traditionally, identification of CWD strains has involved serial passage in animal bioassays (Otero 2022); however, such assays are not well-suited to surveillance activities because they are expensive, time-consuming, and offer limited characterization when strain phenotypes overlap. Other methods of identifying CWD strains (ex vivo and in vitro) have been developed, including PMCA and RT-QuIC assays, but verification using these assays currently requires passage in animals to establish the incubation period, clinical profile, and neuropathology of a specific strain (Benavente 2023, Otero 2022); therefore, these methods on their own are generally not suitable for surveillance, pending regulatory approval (Otero 2022). Improved capabilities for strain characterization and assessment of strain properties are critical to understanding CWD risk over time. Ongoing efforts, therefore, are needed to develop high-throughput and cost-effective methods for CWD strain identification, including further research into the use of amplification

assays for this purpose. Cryogenic electron microscopy holds considerable promise as another plausible way to investigate strain diversity. CWD strain typing at the molecular level is a growing field of study and, based on previous work with rodent-adapted scrapie strains, is expected to expand (Hoyt 2022, Manka 2023). High-resolution CWD prion structures from a naturally infected white-tailed deer have now been reported using cryo-electron microscopy (cryo-EM) (Alam 2024). However, each new prion structure requires complex biochemical purifications and can take many months to solve, meaning few facilities currently have the resources to perform cryo-EM analyses. Without major technological improvements and sufficient facilities, cryo-EM will probably not become a viable routine approach to CWD strain differentiation.

Prion Disease Testing in Non-Cervid Production Animals

Challenges with Diagnosing CWD in Non-Cervid Production Animals

CWD-specific surveillance is not conducted in production animals other than captive cervids. The USDA, however, does conduct surveillance for BSE in cattle and for scrapie in sheep and goats. These surveillance programs employ the Bio-Rad ELISA test kit to detect BSE and scrapie, which is also used to screen for CWD in Canada and in wild cervids in the United States. The Bio-Rad ELISA, however, cannot discriminate between the three prion diseases, and confirmatory testing is necessary to confirm the etiology. A number of challenges exist with confirming the diagnosis of CWD in a non-cervid production animal, including the following:

- Consensus is lacking as to what testing outcomes will provide convincing evidence of a CWD spillover to a non-cervid production animal.
- CWD prions propagating outside their natural host range may behave and manifest quite differently, accumulating in unexpected anatomical regions. For example, if a detection occurred in an infected cow and it was identified through the USDA's current BSE protocol, initial screening and confirmatory testing would be conducted only on the obex. Because the BSE protocol excludes other brain regions, relevant tissues, and body fluids from assessment, anomalous CWD prion deposits may be missed. In addition, previous research has established that different strains of disease-causing prions accumulate differently in the obex region of the host species' brainstem (Lambert 2021, Pirisinu 2018), which may make it more difficult to identify CWD infection in obex tissue.
- If tissue from a CWD-infected cow is tested, the test may lack the sensitivity to identify the spillover incident.
- Distinguishing CWD from BSE or scrapie on confirmatory testing may be difficult. While evidence from experimentally inoculated animals suggests that CWD may have a different WB banding pattern than other TSEs, interpretations are limited by the controlled conditions and small sample size (Greenlee 2012).

An amplification assay may be the best option to detect CWD in a non-cervid animal, assuming there is a substrate capable of showing seeding activity with a novel prion. RT-QuIC and PMCA are not currently USDA-approved CWD tests, but SAAs may be valuable in detecting the emergence of a new prion strain from an interspecies event. At this time, however, SAAs may not be included in testing procedures during an investigation; thus, detection methods will likely primarily rely on ELISA or IHC testing, which cannot measure prion seeding activity or estimate infectivity. SAA technologies can offer evidence of infectious prion contamination in animal housing facilities and theoretically demonstrate the spillover CWD prions' species of origin by comparing the conversion

efficacies of various species substrates (Harpaz 2023, Soto 2025). Continued USDA validation efforts, in coordination with prion researchers, will therefore be essential to integrate SAAs into future investigations and routine CWD surveillance efforts.

Building Laboratory Reference Materials for Spillover Investigations

A major issue that researchers will face while investigating a spillover scenario is determining the availability and utility of appropriate control tissues for comparing test results. Labs that specialize in CWD transmission-challenge studies, such as the USDA's National Animal Disease Center, hold bovine brain tissue from experimentally infected animals for reference material. Looking forward, it will be critical to have tissue from pre-existing experimental studies in bovine hosts as a reference for assessment of any suspected CWD spillover to cattle. A database containing tissue assay results from CWD-inoculated cows, sheep, goats, pigs, and other animals of interest could be a highly valuable resource if a CWD spillover event occurs. Even with the availability of such a database, owing to the unknown pathology of a spillover case, reference tissues may have limited application and utility. For example, a spillover incident would indicate abnormal disease transmission circumstances, and results may not be directly comparable to existing tissue samples, especially if experimental animals were inoculated intracerebrally.

In addition to research in large animals, more work could be accomplished with transgenic mice, considering the space and funds required to perform experiments with cattle and other large animals. For example, transmission through bank voles and cervidized mouse models provided key evidence in determining that CWD in Nordic reindeer and moose differed from CWD in North America (Nonno 2020, Bian 2021). Researchers could subject bovinized mice to known CWD strains in North America and assess the factors that influence transmission. This volume of work is not feasible to conduct in cattle, but transgenic mouse models provide a more practical option. Testing a more comprehensive range of host and recipient species combinations will build confidence in transmission patterns and better characterize disease pathology for a spillover investigation. A table assessing spillover risk by species, acknowledging the considerable number of unknowns and possibility of secondary transmission via an intermediate host, would be a valuable tool in better understanding possible transmission patterns.

Optimizing Diagnostic Strategies for Spillover

Expanding diagnostic options for assessing spillover events through the validation of supplementary tests may be beneficial in developing an optimal testing strategy, yet the distinctive nature of CWD complicates the scenario. For example, developing effective standard operating procedures would have to account for multiple factors such as optimal tissue types, relevant cervid species, *Prnp* genotypes, course of infection, and prion strains. Furthermore, an optimal testing strategy may be laborious and difficult to scale. To expedite a spillover investigation, consideration should be given to developing a work plan for CWD testing in that scenario and establishing the methods that would be used to differentiate CWD in a non-cervid production animal from another TSE.

Key Findings

- No existing test meets all CWD diagnostic needs because they are context-dependent. Used in tandem, they can provide important information; however, test sensitivity early in disease progression may be low, and important knowledge gaps remain.
- Laboratory capacity is a challenge for CWD testing during the hunting season. Existing validated assays are resource-intensive and require expensive equipment, trained personnel, adequate space, and a sufficient supply of reagents.
- While seed amplification assays (e.g., PMCA, RT-QuIC) have significantly advanced research involving CWD and other prions, universal standardization of protocols and reagents and regulatory validation remain substantive challenges.
- There is uncertainty in distinguishing CWD strains, because strain phenotypes overlap. New developments in strain identification methods will likely result in further refinement of strain definitions and classification.
- Although the implications of emerging CWD strains are relevant to the likelihood of interspecies transmission, available information on CWD strain diversity and related dynamics is limited. One reason for this is the small number of tools that can be used for comprehensive, high-throughput strain typing in surveillance settings.
- Experimental transmission studies provide valuable insight into prion disease species barriers, but the generalizability of results from these models/platforms is limited because laboratory conditions may not mimic natural conditions.
- The inability to predict how CWD would present if transmitted to a non-cervid species generates uncertainty about whether current diagnostic tools are sufficient to recognize spillover within the protocols of existing surveillance systems.
- Although available information strongly suggests that existing amplification assays will be useful in CWD spillover investigations, whether they or any other diagnostic assays could detect or discriminate between all possible spillover strains is uncertain.

References

Alam P, Hoyt F, Artikis E, et al. Cryo-EM structure of a natural prion: chronic wasting disease fibrils from deer. Acta Neuropathol. 2024 Oct 24;148(1):56 [Full text]

Bartz JC, Benavente R, Caughey B, et al. Chronic wasting disease: State of the science. Pathogens. 2024 Feb 2;13(2):138 [Full text]

Benavente R, Reed JH, Lockwood M, et al. PMCA screening of retropharyngeal lymph nodes in white-tailed deer and comparisons with ELISA and IHC. Sci Rep. 2023 Nov 17;13(1):20171 [Full text]

Bian J, Kim S, Kane SJ, et al. 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 deer prion protein. PLoS Pathog. 2021 Jul 26;17(7):e1009748 [Full text]

Browning SR, Mason GL, Seward T, et al. Transmission of prions from mule deer and elk with chronic wasting disease to transgenic mice expressing cervid PrP. J Virol. 2004 Dec;78(23):13345-50 [Full text]

Burgener KR, Lichtenberg SS, Lomax A, et al. Diagnostic testing of chronic wasting disease in white-tailed deer (*Odocoileus virginianus*) by RT-QuIC using multiple tissues. PLoS One. 2022 Nov 16;17(11):e0274531 [Full text]

Canadian Food Inspection Agency. Chapter 13 - Chronic Wasting Disease Herd Certification Programs. 2020 [Web page]

Cook M, Hensley-McBain T, Grindeland A. Mouse models of chronic wasting disease: A review. Front Virol. 2023 Feb 19;3 [Full text]

CWD Research Consortium. Research Priorities White Papers. 2021 Mar [Web page]

Case Western Reserve University. Autopsy Testing Reporting. 2024 [Web page]

Case Western Reserve University. Brain Magnetic Resonance Imaging (MRI). 2024 [Web page]

Case Western Reserve University. National Prion Disease Pathology Surveillance Center Testing and Reporting Policies. 2024 [Web page]

Darish JR, Kaganer AW, Hanley BJ, et al. Inter-laboratory comparison of real-time quaking-induced conversion (RT-QuIC) for the detection of chronic wasting disease prions in white-tailed deer retropharyngeal lymph nodes. J Vet Diagn Invest. 2024 Oct 14:10406387241285165 [Full text]

Figgie MP Jr, Appleby BS. Clinical use of improved diagnostic testing for detection of prion disease. Viruses. 2021 Apr 28;13(5):789 [Full text]

Greenlee JJ, Nicholson EM, Smith JD, et al. Susceptibility of cattle to the agent of chronic wasting disease from elk after intracranial inoculation. J Vet Diagn Invest. 2012 Nov;24(6):1087-9 [Full text]

Harpaz E, Vuong TT, Tran L, et al. Inter- and intra-species conversion efficacies of Norwegian prion isolates estimated by serial protein misfolding cyclic amplification. Vet Res. 2023 Sep 29;54(1):84 [Full text]

Hibler CP, Wilson KL, Spraker TR, et al. 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*). J Vet Diagn Invest. 2003 Jul;15(4):311-9 [Full text]

Holper S, Lewis V, Wesselingh R, et al. Comprehensive clinical, radiological, pathological and biochemical analysis required to differentiate VV1 sporadic Creutzfeldt-Jakob disease from suspected variant CJD. BMJ Neurol Open. 2022 Apr 18;4(1):e000299 [Full text]

Holz CL, Darish JR, Straka K, et al. Evaluation of real-time quaking-induced conversion, ELISA, and immunohistochemistry for chronic wasting disease diagnosis. Front Vet Sci. 2022 Jan 18;8:824815 [Full text]

Hoyt F, Alam P, Artikis E, et al. Cryo-EM of prion strains from the same genotype of host identifies conformational determinants. PLoS Pathog. 2022 Nov 7;18(11):e1010947 [Full text]

Lambert ZJ, Greenlee JJ, Cassmann ED, et al. Differential accumulation of misfolded prion strains in natural hosts of prion diseases. Viruses. 2021 Dec 7;13(12):2453 [Full text]

Manka SW, Wenborn A, Betts J, et al. A structural basis for prion strain diversity. Nat Chem Biol. 2023 May;19(5):607-13 [Full text]

Marsh RF, Kincaid AE, Bessen RA, et al. Interspecies transmission of chronic wasting disease prions to squirrel monkeys (*Saimiri sciureus*). J Virol. 2005 Nov;79(21):13794-6 [Full text]

Mathiason CK, Powers JG, Dahmes SJ, et al. Infectious prions in the saliva and blood of deer with chronic wasting disease. Science. 2006 Oct 6;314(5796):133-6 [Full text]

Mazza M, Tran L, Loprevite D, et al. Are rapid tests and confirmatory western blot used for cattle and small ruminants TSEs reliable tools for the diagnosis of chronic wasting disease in Europe? PLoS One. 2023 Aug 30;18(8):e0286266 [Full text]

McNulty E, Nalls AV, Mellentine S, et al. Comparison of conventional, amplification and bio-assay detection methods for a chronic wasting disease inoculum pool. PLoS One. 2019 May 9;14(5):e0216621 [Full text]

Nonno R, Di Bari MA, Pirisinu L, et al. Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America. Proc Natl Acad Sci USA. 2020 Dec 8;117(49):31417-26 [Full text]

Olszowy KM, Lavelle J, Rachfal K, et al. Six-year follow-up of a point-source exposure to CWD contaminated venison in an Upstate New York community: Risk behaviors and health outcomes 2005-2011. Public Health. 2014 Sep;128(9):860-8 [Full text]

Otero A, Duque Velasquez C, McKenzie D, et al. Emergence of CWD strains. Cell Tissue Res. 2022 Oct 6;392(1):135-48 [Full text]

Picasso-Risso C, Schwabenlander MD, Rowden G, et al. 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. 2022 Apr 20;11(5):489 [Full text]

Pirisinu L, Tran L, Chiappini B, et al. Novel type of chronic wasting disease detected in moose (*Alces alces*), Norway. Emerg Infect Dis. 2018 Dec;24(12):2210-8 [Full text]

Rowden GR, Picasso-Risso C, Li M, et al. Standardization of data analysis for RT-QuIC-based detection of chronic wasting disease. Pathogens. 2023 Feb 13;12(2):309 [Full text]

Sajnani G, Requena JR. Prions, proteinase K and infectivity. Prion. 2012 Nov-Dec;6(5):430-2 [Full text]

Seelig DM, Mason GL, Telling GC, et al. Chronic wasting disease prion trafficking via the autonomic nervous system. Am J Pathol. 2011 Sep;179(3):1319-28 [Full text]

Schneider DA, Lehmkuhl AD, Spraker TR, et al. Tonsil biopsy to detect chronic wasting disease in white-tailed deer (*Odocoileus virginianus*) by immunohistochemistry. PLoS One. 2023 Mar 30;18(3):e0282356 [Full text]

Soto P, Bravo-Risi F, Benavente R, et al. Detection of prions in wild pigs (*Sus scrofa*) from areas with reported chronic wasting disease cases, United States. Emerg Infect Dis. 2025 Jan;31(1) [Full text]

Sun JL, Telling GC. New developments in prion disease research using genetically modified mouse models. Cell Tissue Res. 2023 Apr;392(1):33-46 [Full text]

US Department of Agriculture Animal and Plant Health Inspection Service. BSE Surveillance Information Center. 2024 [Web page]

US Department of Agriculture Animal and Plant Health Inspection Service. National Scrapie Surveillance Plan. 2022 Aug [Full document]

US Department of Agriculture Animal and Plant Health Inspection Service. Chronic Wasting Disease Program Standards. 2019 [Full document]

US Department of Agriculture Animal and Plant Health Inspection Service Veterinary Services. Annual Update from the Cervid Health Team Fiscal Year (FY) 2019 [Full document]

Wappes J. US experiencing nationwide shortage of tests for chronic wasting disease. CIDRAP News. 2023 Feb 2 [Web page]

Watts JC, Prusiner SB. Mouse models for studying the formation and propagation of prions. J Biol Chem. 2014 Jul 18;289(29):19841-9 [Full text]

Chapter 3: Spillover to Non-Cervid Production Animals: Surveillance, Laboratory Capacity, Planning, and Response

Introduction

CWD has been experimentally transmitted to non-cervid animals, although instances of naturally occurring CWD transmission to non-cervid species have not been identified. Natural CWD transmission to a non-cervid animal would signal that CWD can cross species barriers and establish a new host range. Transmission to non-cervid production animals (e.g., cattle, goats, sheep, pigs) is of particular concern because of the associated economic, food safety, human health, and environmental consequences. This chapter focuses on issues associated with spillover to non-cervid production animals. The evidence demonstrates a growing need for targeted, standardized research to address knowledge gaps.

Surveillance

BSE and Scrapie Surveillance

CWD-specific surveillance is not conducted in production animals other than captive cervids. The USDA, however, conducts surveillance for BSE in cattle and for scrapie in sheep and goats. Prion disease surveillance is not conducted in other production animals, such as pigs, because prion disease has not been identified in those species. The USDA BSE surveillance system samples about 25,000 animals each year, with most samples coming from facilities that render animal carcasses after slaughter, a small percentage coming from animals with abnormalities that could be associated with BSE, such as neurological disease, and the rest from farms, veterinary facilities, and livestock markets (USDA). The CFIA operates under a very similar surveillance model (CFIA 2024). Carcasses from sampled animals intended for further processing are required to be held until laboratory results

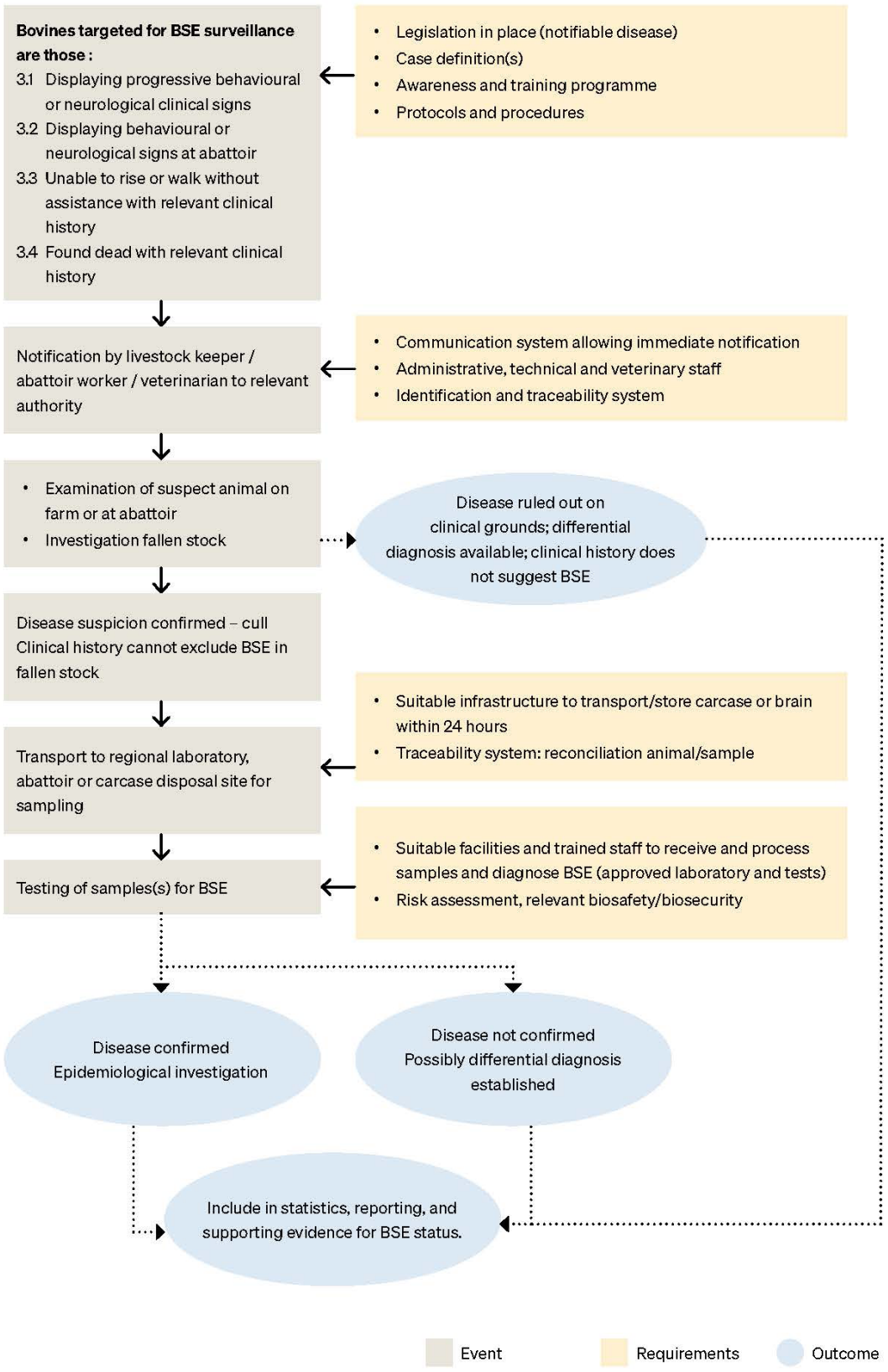


Figure 5: WOAH's Overview of Surveillance System Components to Detect BSE, Figure A (WOAH 2023)

are received. The World Organization for Animal Health's (WOAH's) flowchart on the components of a credible surveillance system for BSE could serve as a model for testing recommendations following detection of a suspected positive CWD case in a non-cervid production animal (Figure 5) (WOAH 2023).

The USDA and CFIA also each have a national scrapie surveillance plan aimed at scrapie eradication, and they prioritize testing sheep and goats on farms (targeting high-risk groups), at slaughter (animals with clinical signs and a sample of healthy animals), and during disease investigations (USDA 2022, CFIA 2023). Outside of these routine surveillance efforts, herd veterinarians across the country are urged to report and test production animals with clinical signs consistent with prion disease. Given the important role that veterinarians have in disease detection, expanding educational opportunities that enhance awareness and recognition of prion diseases is encouraged.

Surveillance Challenges

Because CWD may manifest differently in non-cervid production animals, existing surveillance programs for BSE and scrapie could misdiagnose a case of CWD spillover in cattle, sheep, or goats. For example, the BSE surveillance program performs confirmatory testing only on the obex. If CWD prions accumulate differently in the obex or primarily in other cattle brain regions or tissues, the diagnosis may be missed, as may happen with atypical forms of BSE. Also, it may be difficult to distinguish CWD prions from BSE or scrapie using only WB. To address some of these concerns, the USDA BSE or scrapie protocol could be modified to require that a range of tissue samples from animals with suspected disease be preserved and made available for later testing as needed (USDA 2022). One practical strategy to accomplish this would be to require that facilities hold the entire carcass while prion disease testing is underway. Because BSE testing involves initial screening by ELISA, further experimental studies are needed to establish ELISA optical density trends and baselines on tissues from cervids with CWD, cattle with BSE, and cattle with CWD to inform how best to distinguish CWD and BSE in cattle using a screening platform.

Because prion disease surveillance is not conducted in animals other than cattle, sheep, and goats, identifying CWD in other populations poses additional challenges. For example, experimental evidence indicates that swine are susceptible to prion infection following oral or intracranial inoculation (Moore 2017). In addition, a recent study confirmed interactions between CWD prions and feral swine in natural settings (Soto 2025). However, prion disease is not routinely considered in swine, and CWD spillover would probably be missed. Furthermore, officials have not determined the regulatory and disease-reporting implications of a CWD detection in swine or poultry.

Investigation of a Suspected Spillover Incident

The response to a suspected CWD case in a non-cervid production animal would require both epidemiologic and laboratory investigations. The combined priorities of these investigations include determining the origin of infection, identifying other at-risk animals, assessing risk to humans, and characterizing transmission pathways and disease manifestation in the novel host.

Epidemiologic and Environmental Investigations

If a CWD case is identified in a non-cervid production animal, epidemiologic investigation and traceback will be critical to determine (1) where and how the animal acquired the infection and (2) whether other animals or humans are at risk for transmission. Although an epidemiologic investigation is crucial, several barriers hinder its comprehensive execution.

- Animal movement is a known risk for infectious disease transmission, as elucidated by the 2024 spillover of H5N1 avian influenza virus into cattle across the United States (AVMA 2024) and the introduction of CWD into captive cervid herds through the introduction of animals from farms later found to be CWD-positive (Kincheloe 2021). Cattle may be transported to multiple facilities and states before slaughter, complicating identification of the initial exposure and increasing the number of secondarily exposed animals.
- Records of animal location history will be useful for traceback efforts. In the United States, such records are often incomplete, and even if available, significant resources are required to obtain relevant data from all locations. However, as of November 2024, the USDA mandates the use of radio frequency identification (RFID) ear tags in certain populations of cattle and bison to improve traceability (Federal Register 2024). Canada has required RFID tags for disease control since 2002, with measurable traceability effectiveness during disease outbreak investigations (Canadian Cattle Association).
- Depending on information obtained through the traceback process, investigators may need to work with producers at locations around the country to cull and sample additional animals. Disease control measures, including culling animals for testing, may be necessary to understand the extent of the CWD spillover, although the scale of such measures will need to be carefully considered. Such efforts will likely be complicated by confidentiality issues that will limit the information that can be shared between agencies.
- Local CWD surveillance in cervid populations is highly variable, often underestimates disease prevalence, and lacks strain typing, leaving uncertainty about how infected wildlife or contaminated pastures may have contributed to spillover.
- In the unlikely event that one site is identified as the source of the spillover, confirmation will likely result in pressure on wildlife agencies to reduce or eliminate wild cervids from the environment surrounding the facility. In this scenario, public and political skepticism may arise surrounding management of free-ranging deer and other cervids.
- Environmental sampling of the infected animal's current and past enclosures/pastures for CWD prions would aid the understanding of how the animal was exposed and in determining prion contamination of the immediate environment. However, responders may not be able to access the area for testing without the producer's permission, which they may be reluctant to grant without clear USDA guidance, fearing personal or legal consequences. This barrier will present major challenges to characterizing the transmission of CWD to a non-cervid production animal.
- If prions are in the environment, locating contamination sources may be challenging if they are not ubiquitous. For example, if the CWD prions came from a wild cervid on a free-ranging cattle pasture, widespread evidence of environmental contamination may not be present; rather, the source may be localized to a small area that may not be detected. If CWD prions are found through extensive environmental sampling, additional tests will need to characterize the environmental prion load and transmissibility to non-cervid species.

Laboratory Investigation

Beyond detection and confirmatory diagnosis, laboratory investigation will be needed to characterize disease manifestation in the novel host and implications for transmission. In the instance of interspecies prion transmission, pathological features in the newly infected animal could be different from the typical presentation in cervids. Examining and testing several extraneural peripheral tissues, feces, blood, and urine during necropsy will be important to determine whether the animal could have shed prions in body fluids or excreta. For example, the presence of prions in the peripheral lymph nodes would indicate that the infection closely resembles that of scrapie in sheep and goats and CWD in cervids. This finding would mean that the infected animal was more likely to shed prions, increasing the possibility of direct horizontal transmission and shedding of prions into the environment. The presence or absence of prions in the peripheral lymph nodes would be critical evidence to inform the traceback investigation and response strategy.

A tissue of high consequence for CWD prion detection in a non-cervid production animal is skeletal muscle, because consumption of muscle tissue could be a source of direct alimentary exposure to humans. CWD prions have been found in the muscle tissue of infected cervids; therefore, if spillover were to occur, it will be crucial that preserved muscle samples from infected animals are archived and made available to include in the diagnostic flow as part of the overall risk assessment. Evaluations of animals with naturally occurring BSE, however, suggest that the highest concentration of prions is localized to the central nervous system (CNS), with limited evidence of peripheral spread to muscle tissue (CFSPH 2016). Therefore, eating meat and animal products contaminated with CNS tissue poses a risk of prion transmission to humans, even with limited direct skeletal muscle involvement (EFSA 2024).

Expected Protocol for Evaluating an Index CWD Spillover Case in a Production Animal

An established workflow or testing protocol does not exist for investigating a potential CWD spillover to a non-cervid animal. The paragraphs below, however, outline steps that officials would likely follow if CWD were suspected in a non-cervid production animal—cattle in this case. A similar process would be followed for sheep and goats. Because surveillance is not performed in other production animals, the diagnostic workflow is less clear, although it will probably follow a similar general approach.

Stage 1: Initial detection

Assuming that CWD results in similar neurologic signs in bovines as in cervids, a spillover event in a bovine could be detected through the USDA BSE surveillance program or, if outside that system, could be recognized and reported to the USDA by the herd's veterinarian. In particular, cattle used for breeding may be an important group for surveillance because they have a longer lifespan in both the beef and dairy cattle industries and, therefore, may be most likely to develop clinical disease. In either case, a veterinarian would likely order a screening test for BSE. As mentioned previously, the Bio-Rad ELISA is used as the screening test and can detect scrapie, BSE, and CWD prions but cannot discriminate between them.

Stage 2: Confirmatory testing

If a screening test from an animal with an unidentified neurological disease is positive for prions, all available samples would be forwarded to the NVSL for further testing and analysis using WB and IHC. Based on data published by researchers at the USDA's Agricultural Research Service, WB profiles of cattle infected with BSE can be distinguished from those of cattle infected with CWD (Greenlee 2012). Furthermore, the researchers examined PrP immunoreactivity patterns with IHC and found different localization and

intensity of PrP^{Sc} deposition in the CNS of the two cattle infected with CWD from those in cattle infected with BSE. These results suggest that confirmatory testing with WB and IHC should identify differences between CWD and BSE in a bovine (although it is more challenging to distinguish between L-type BSE and CWD in a bovine using WB). Scientists, therefore, expect the WB banding pattern of CWD to differ from that of BSE, although the WB banding pattern of CWD may depend on the CWD prion strain involved in the transmission (Marín-Moreno 2024). Questions remain, however, as to whether confirmatory testing will be able to differentiate CWD from BSE in an affected animal. For example, studies of CWD transmission from cervids to bovine have involved intracerebral inoculation, which has been linked to differentiation of prion strain properties such as PrP^{Sc} conformational stability, sensitivity to the enzyme proteinase K, and migration patterns on sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE; medium typically used in WB) (Otero 2023). It is unclear what the WB profile would look like for PrP^{Sc} transmitted via the oronasal route compared with that of prions from wild cervids or administered to bovines intracerebrally, in part because oral challenge studies have been relatively unsuccessful at disease transmission to date (Hamir 2005, Williams 2018). Without knowing the unique biochemical markers or pathological properties that will be apparent in confirmatory tests, a CWD spillover case could be misdiagnosed as BSE or remain unidentified. At the laboratory level, any unusual PrP^{Sc} WB banding pattern or something of equal concern would lead to a large-scale discussion among several groups associated with the NVSL. Considering the economic implications to agricultural industries, agencies likely will face data-sharing challenges.

Stage 3: Testing of additional tissues and body fluids

Regulatory agencies will need high confidence in the diagnosis because of the significant implications of a CWD spillover event and the importance of initiating risk communication activities. At this stage, it will be critical to have a risk communication plan in place and available for implementation. If testing at the NVSL points to an atypical prion disease other than BSE, researchers will likely opt to perform additional tests, so extraneural tissues should be collected and available for testing in the diagnostic flow. The NVSL laboratory should test a variety of brain tissue areas, because the spillover prion strain may be concentrated in an unconventional region of the brain. In addition, investigators should collect and test body fluids from the infected animal to identify any shedding pattern that could have led to exposure in cohabitating cattle.

Stage 4: Further investigation to determine infectivity

Assuming that sufficient evidence exists to classify the atypical prion disease case in a bovine as CWD, the USDA will initiate a larger investigation to determine the route and mechanism of the spillover incident and, in collaboration with the Centers for Disease Control and Prevention (CDC), the risk of transmission to humans. In early stages, the investigation will likely resemble the BSE Response Plan and remain under federal jurisdiction. To determine whether the spillover CWD strain poses a greater risk of zoonotic transmission if found in a production animal, transmission studies in cervid PrP^{C} knock-in or overexpressing mice or NHPs will be needed, although these approaches require a significant investment of time and resources. Such animal bioassays are important for detecting infectious prions in the tissues and determining their anatomic distribution. Because the USDA has not approved SAAs for regulatory use as of September 2024, PMCA or RT-QuIC may not be readily available as part of a spillover-investigation testing protocol. This barrier prevents collecting additional evidence about infectivity that these assays could provide. Given the gravity of the situation, all available approaches will be important, and highly

detailed, long-term studies would ideally be funded shortly after the incident. Research into zoonotic transmission potential from a CWD-infected production animal would be required before any changes are made to human diagnostic procedures.

Stage 5: Further characterization of the spillover event

Further research will be needed to determine how the prion strain evolved to cross the species barrier. Access to CWD-infected samples from wild cervids in the same geographic region before the spillover event will be critical for this analysis. This will require studying the genome of the infected animal to determine if the animal had any known genetic susceptibility to prion disease or had a new genomic variant that increased susceptibility to CWD prions (Otero 2021, Mysterud 2023). A spillover event into cattle will lead to many new investigations into CWD pathogenesis, involvement of maternal transmission, and other mechanisms that may aid in identifying future cases. Findings from ongoing CWD research in cervids may also inform investigation efforts, although more work is needed to better understand the mechanisms of prion transport into the host's bloodstream, central nervous system, and lymphatic tissue. One way to address these challenges is to establish an incident advisory group of prion biology experts that could work alongside the NVSL diagnostic team to support the investigation. The NVSL could share relevant tissue samples with academic research groups to investigate using their own standard operating procedures. Required laboratory permits should be obtained in advance.

Regulatory Action

Pre-Spillover Incident

Proactive regulatory action can aid the epidemiologic and laboratory investigation of a CWD-infected non-cervid production animal. For example, improving the electronic tagging system for production animals will ease the traceability of animals of interest in a spillover investigation. In addition, policies outlining testing recommendations should be outlined pre-spillover, to the extent possible, so that protocols can be developed and implemented immediately if and when an investigation is initiated.

Post-Spillover Incident

Little will be known about the transmissibility and shedding capacity of a particular CWD strain after it infects a novel host, leaving many questions about biosecurity protocols for other animal contacts and for the facilities where the infected animal was housed and processed. Regulatory authorities will be responsible for interpreting the results of the epidemiological and laboratory investigations to enact and implement policies that protect the safety of products entering the food supply and limit collateral costs to owners of non-cervid production animals.

Regulatory actions will also need to consider how the response to a suspected or confirmed CWD case in a non-cervid production animal will affect trade. Trade restrictions put in place by countries such as Norway, which banned the import of hay and straw from CWD-positive states and provinces, serves as evidence that trade repercussions are possible for products from CWD-affected areas (Van Beusekom 2024). Authorities will want to avoid unnecessarily jeopardizing trade by being cautious in the interpretation of preliminary results indicating that an animal may have developed CWD, but the confirmation process may require significant time. The response of the agriculture and non-cervid production animal industries will be another consideration. These industries may question regulatory recommendations by arguing that the ongoing consumption of CWD-positive cervids is a more pressing issue. Authorities need to anticipate such questions and develop a response strategy to be able to implement successful recommendations and policies to protect food safety.

Communication and Collaboration

The current communications landscape for potential CWD spillover information lacks cohesion. Processes for sharing information with the public can vary among agencies and jurisdictions. Differences may include delivery methods, timing of announcements, and level of detail. The USDA has potential resources for communication and messaging development via a One Health group assembled owing to concerns about COVID-19 spillover. Framing CWD as a One Health concern engages various federal partners to help address the issue as a larger group. An incident command system within the federal government also has infrastructure to coordinate the federal response and communicate consistent messaging across federal agencies, including the CDC. Experts and stakeholders recommend incorporating public health, animal health, and agriculture expertise when developing CWD spillover response materials.

Following a spillover, public messaging should include information on what constitutes a confirmed spillover case, the safety of meat in the food supply, and occupational safety issues. Producers will want additional information about testing, movement restrictions, and how their operations will otherwise be impacted. Owners of production facilities will need information on the long-term environmental implications of prion persistence in their facilities and decontamination. Communication materials from previous livestock-related infectious disease outbreaks may be useful templates for the CWD spillover response.

Research

Laboratory versus Natural Setting Research

Controlled experiments involving natural cervid hosts can offer valuable scientific insights, but few groups have the capacity to conduct such research because of high costs, lack of adequate space, and the required expertise. Conversely, transgenic mouse models are less expensive and more accessible for laboratories to use. However, results from such experiments may be less generalizable to the natural environment. For example, certain lines of transgenic mice overexpress levels of normal prion protein (PrP^C), and inoculation routes commonly used in the laboratory setting (e.g., intracranial inoculation) do not reflect natural transmission routes. In addition, in both natural cervid hosts and transgenic animal models, studies can take months or years to generate results, owing to the slow progression of disease.

Research Challenges

Research to inform diagnostic decision making is needed to explore which non-cervid species may be susceptible to CWD infection and how the disease would manifest in novel hosts. Efforts to date to determine various hosts' susceptibility have been limited by several factors. For example, because the minimum infectious dose of CWD prions is not known, infective prion dosages used in experiments vary among studies and laboratories. Inoculation routes, exposure type (direct vs. indirect), and exposure frequency also vary. Titered inoculations of CWD material into animals of interest via various transmission pathways with other standardized experimental variables will be useful for replication. A defined source of standardized CWD reference strains is also needed, because strain behavior, including zoonotic potential, can differ.

Research Priorities

Despite the challenges, research is urgently needed to answer critical questions about the potential of CWD transmission to non-cervids and disease manifestation in a novel host. Specifically, large-animal studies, ideally using different strains of CWD prions, should be performed to explore the clinical signs, tissue distribution, and shedding potential of CWD prions in a non-cervid production animal host when infected through oral-nasal exposure.

Testing a more comprehensive range of host and recipient species combinations will build confidence in transmission patterns and help characterize disease pathology in a suspected case. A specimen bank containing tissue from CWD-inoculated cattle, sheep, pigs, and other animals will be an important reference for assessment of a suspected CWD spillover. Experiments with transgenic mice subjected to known CWD strains in North America can help identify the factors (e.g., genetic) that influence transmission, as long as the results are interpreted with caution.

Key Findings

- Prion disease surveillance is limited or absent for most production animals. Although scrapie and BSE surveillance programs are in place for cattle, goats, and sheep, they are insufficient to identify a CWD spillover in all production animals.
- No single test can identify CWD transmission to a non-cervid species, clearly presenting a major diagnostic challenge. In theory, a combination of existing tests could be used, but data are not available on whether they could differentiate BSE or scrapie from CWD in a novel animal host.
- CWD strains likely differ in virulence, transmissibility, and zoonotic potential. However, CWD strains are poorly characterized, agreement on what constitutes a strain is lacking, and easy methods for comprehensive strain typing are not available.
- Following a spillover event in which CWD infects a novel host species, the pathological features of disease (e.g., prion distribution, lymphotropism, prion shedding capacity) could be different than those observed in cervids. Determining these differences is essential to evaluate transmission risk, inform traceback investigations, and determine response strategies (including biosafety guidelines).
- Response to a CWD spillover is complicated because multiple agencies have jurisdictional authority, and detailed contingency plans for interagency cooperation do not exist. Furthermore, regulatory and logistical barriers to access premises for spillover investigations present additional challenges.
- The roles of external academic centers and laboratories during a CWD spillover investigation are undefined, although they can offer essential expertise.
- Regulatory authorities will need to anticipate and plan contingencies for the impact of CWD management on trade policies and production animal and agriculture industries.
- Although precedents exist for agencies to scale up testing in response to disease spillover (e.g., H5N1, COVID-19, bovine tuberculosis), CWD poses unique challenges owing to costs, space demands, and other testing-method unknowns (i.e., some tests are more labor intensive, slower, or unvalidated).

References

American Veterinary Medical Association. Avian influenza virus type A (H5N1) in US dairy cattle. 2024 Sep 26 [Web page]

Canadian Cattle Association. Standards and Practices. [Web page]

Canadian Food Inspection Agency. BSE enhanced surveillance program. 2024 [Web page]

Canadian Food Inspection Agency. National Scrapie Eradication Program. 2023 [Web page]

Center for Food Security & Public Health. Bovine Spongiform Encephalopathy. 2016 Aug [Full document]

European Food Safety Authority. Bovine spongiform encephalopathy (BSE). 2024 July 16 [Web page]

Federal Register. Use of Electronic Identification Eartags as Official Identification in Cattle and Bison. United States National Archives. 2024 May 9 [Full document]

Greenlee JJ, Nicholson EM, Smith JD, et al. Susceptibility of cattle to the agent of chronic wasting disease from elk after intracranial inoculation. J Vet Diagn Invest. 2012 Nov;24(6):1087-93 [Full text]

Hamir AN, Kunkle RA, Cutlip RC, et al. Experimental transmission of chronic wasting disease agent from mule deer to cattle by the intracerebral route. J Vet Diagn Invest. 2005 May;17(3):276-81 [Full text]

Kincheloe JM, Horn-Delzer AR, Makau DN, et al. Chronic wasting disease transmission risk assessment for farmed cervids in Minnesota and Wisconsin. Viruses. 2021 Aug 11;13(8):1586 [Full text]

Marín-Moreno A, Benestad SL, Barrio T, et al. Classical BSE dismissed as the cause of CWD in Norwegian red deer despite strain similarities between both prion agents. Vet Res. 2024 May 15;55(1):62 [Full text]

Moore SJ, West Greenlee MH, Kondru N, et al. Experimental transmission of the chronic wasting disease agent to swine after oral or intracranial inoculation. J Virol. 2017 Sep 12;91(19):e00926-17 [Full text]

Mysterud A. An epidemiological framework for chronic wasting disease in multihost communities. J Appl Ecol. 2023 Jun 1;60(8):1545-50 [Full text]

Otero A, Velásquez CD, Aiken J, et al. Chronic wasting disease: a cervid prion infection looming to spillover. Vet Res. 2021 Sep 6;52(1):115 [Full text]

Otero A, Velásquez CD, McKenzie D, et al. Emergence of CWD strains. Cell Tissue Res. 2023 Apr;392(1):135-48 [Full text]

Soto P, Bravo-Risi F, Benavente R, et al. Detection of prions in wild pigs (*Sus scrofa*) from areas with reported chronic wasting disease cases, United States. Emerg Infect Dis. 2025 Jan;31(1) [Full text]

US Department of Agriculture. BSE Surveillance Information Center [Web page]

US Department of Agriculture. National Scrapie Surveillance Plan. 2022 Aug [Full document]

Van Beusekom M. Plants can take up CWD-causing prions from soil in the lab. What happens if they are eaten? CIDRAP News. 2024 Feb 22 [Web page]

Williams ES, O'Toole D, Miller MW, et al. Cattle (*Bos taurus*) resist chronic wasting disease following oral inoculation challenge or ten years' natural exposure In contaminated environments. J Wildl Dis. 2018 Jul;54(3):460-470 [Full text]

World Organisation for Animal Health. Guidelines for Targeted BSE Surveillance. 2023 Jul [Full document]

Chapter 4: Environmental Implications of Carcass and Contaminated Item Disposal

Introduction

An element of CWD management that cannot be overlooked involves the disposal of carcasses—including carcass parts, butcher remains, and scraps—and other prion-contaminated items. Prions are extremely stable and can remain infectious in environmental settings for very long periods, at least on the order of years to decades (Brown 1991, Georgsson 2006, Somerville 2019). Thus, when CWD prions enter the environmentvia body fluids shed by an infected animal or contaminated carcass parts deposited on the landscape, any associated risks are likely long-term. This marked persistence, coupled with the highly contagious nature of CWD among cervids, presents expanding opportunities for indirect horizontal transmission as prions accumulate in the environment. Knowing this, a number of regulatory agencies overseeing wild and captive cervid populations have sought to mitigate associated risks by establishing protocols for the disposal of potentially CWD-infected carcasses, including the use of methods such as alkaline digestion, incineration, landfilling, onsite burial, and composting. This chapter provides an overview of CWD prions in the environment, examines the approaches used for cervid carcass disposal, and presents associated challenges relevant to potential spillover.

Current Implications of Environmental Contamination

CWD Prion Environmental Persistence and Fate

CWD prions have been shown to retain infectivity in environmental settings for at least 15 years (Stuart Lichtenberg, personal communication). However, characteristics of these agents are ultimately shaped by complex and mostly uncharacterized networks of biological (e.g., prion strain characteristics) and environmental (e.g., soil composition and pH, precipitation, temperature) factors (Bartelt-Hunt 2023, Baune 2023). Nevertheless, research in this area has provided valuable insight into prion persistence and fate in the environment. For example, data demonstrate that soil type can influence prion transport

and, in some cases, infectivity (Davies 2009, Johnson 2006, Johnson 2007, Saunders 2009), while other factors such as precipitation (e.g., cycles of soil wetting and drying) may reduce persistence (Yuan 2015). While prion concentrations diminish over time, residual infectivity presumably can persist for many years, an important consideration when assessing options for disposal of CWD-contaminated material.

Environmental exposure likely plays an important role in disease incidence, particularly over time as prion density in the environment increases with disease prevalence (Pritzkow 2021) . However, the estimated impact of environmental exposure on the CWD transmission cycle has not been quantified for wild cervids and represents a large gap in assessment of the mitigation of CWD spread; researchers cannot measure the proportion of incident CWD cases attributed to indirect (i.e., environmental) versus direct (i.e., animal-to-animal) transmission.

Environmental Remediation

Prions can persist despite extremely harsh conditions, and this durability limits the methods available to deactivate them. Little can be done to alter the natural environment for CWD management in free-ranging cervids because prions are not ubiquitous in the environment; remediation efforts would have to be very specific. Data suggest that different soil and plant types influence prion persistence and that, depending on soil type, prions can migrate from surface soils into lower soil horizons (Kuznetsova 2023). Soil amendments, or materials added to improve physical or chemical properties, may further alter prion persistence and movement. Ongoing research is exploring how high-heat, high-moisture environments might affect the viability of prions. However, differences may exist in the environmental stability of unique prion strains, making it difficult to extrapolate findings (Bartz 2021, Holec 2019, Maddison 2010).

The USDA's HCP Standards include guidelines on limiting further spread of CWD from contaminated facilities and decontamination procedures, with recognition that there are currently no means of achieving complete environmental remediation (USDA 2019). Foremost, the general approach to biosecurity is to prevent direct and indirect contact between free-ranging cervids and CWD prions. Facilities are required to maintain perimeter fencing, divert wildlife interaction (i.e., among wild cervids, scavengers, and foragers), and minimize outside contact with potentially contaminated objects or materials. Decontamination approaches are aimed at locations in a facility most likely to harbor CWD prions, such as pastures or holding areas. For carcasses from a CWD-positive, suspected, or exposed animal or herd, officials recommend using appropriate disposal approaches such as incineration or alkaline digestion to destroy the carcass, as well as burial at an appropriate depth to isolate the prions and prevent scavenging and groundwater contamination. Some surfaces can be cleaned with an alkaline solution after removing organic material. The USDA recommends against repopulating the facility with CWD-susceptible species, because the decontamination measures are meant to reduce environmental prion burden but not remediate the area.

Applying Diagnostic Tests for Disposal

As discussed in previous chapters, the tests currently approved for diagnosing CWD can effectively determine if an animal has the disease; however, these tests have notable limitations when it comes to informing disposal practices. For instance, while positive test results indicate that prions are present in analyzed tissues (e.g., RPLN, obex), they do not provide a complete assessment of where prions are distributed throughout the body. In addition, although test results could theoretically inform appropriate disposal actions, prolonged turnaround times—often a week or longer—restrict their utility. By the time results are available, many

hunter-harvested cervids have already been processed, and the unused carcass remains are left over. Because these remains are typically present when the animal's disease status has not yet been determined, disposal guidance tends to be precautionary and assumes the animal is infected. While this approach may help mitigate the risk of CWD spread by reducing the number of potentially infected carcasses accessible across the landscape (although evidence of reduction in cervid-to-cervid transmission is deficient), it requires significant resources and lacks precision.

Advances in diagnostics, primarily SAAs such as RT-QuIC and PMCA, have improved the understanding of prion distribution in tissues from an infected animal host because they offer highly sensitive detection, which is particularly important when prion concentrations are low. In recent years, researchers have begun investigating how this technology may inform how prions move through environmental matrices, possibly providing insight on migration through settings such as landfills. However, SAAs may not consistently detect CWD prions in large volumes of water, soil, or other organic material because prion concentrations can vary within a matrix, requiring extensive and careful sampling. Certain soil types and other matrices also inhibit the SAA reaction to varying degrees (Saunders 2011). Therefore, even if prions are not detected in environmental samples, negative SAA results do not guarantee the absence of infectivity (Henderson 2015). In addition, researchers have not identified a concentration-based relationship between detection of prions in the environment and infectious dose. For example, if CWD prions are found at relatively low concentrations in environmental samples, those results cannot necessarily be interpreted as "low-risk" because current research has not established the threshold at which a prion concentration can result in infection. In the future, SAAs may be applied more widely to assess the implications of CWD prion detection in natural and disposal settings, but standardized, thorough methodologies are needed.

Current Carcass Disposal Options, Challenges

Disposal Options

The most commonly used disposal method for hunter-harvested carcasses and byproducts is solid waste landfills because of their relatively low cost and capacity to handle high volumes of waste. Among state wildlife agencies that employ carcass disposal, 84% rely on approved municipal solid waste landfills (Anderson 2023). These sites typically must be licensed subtitle D landfills, which feature clay liners, geomembranes, and leachate- and gas-collection systems that aim to safeguard the surrounding environment from contaminants, including prions.

Onsite burial, in which the remains are buried in the same location where the animal died, is an alternative for hunters and captive-cervid farmers, if allowed by state authorities. Such burial practices often include depth requirements intended to limit continued CWD exposure to wildlife or other farm inhabitants.

Alternative methods for carcass and contaminated-item disposal are incineration and alkaline hydrolysis. Incineration of carcasses using an incinerator with an emissions control system is an effective tool when used properly, but several barriers limit its widespread application for prion-infected carcasses. The temperature required to eliminate prion infectivity is extremely high; the chamber must reach at least 900 C° (1,652° F) (Saunders 2008). Inadequate combustion temperatures (≤600 C° or 1,112° F) may lead to selection of more durable CWD prion strains and allow further disease spread (Saunders 2008). Incineration is also far less accessible than landfilling, and only a small number of state-run departments own and consistently operate these machines.

Like incineration, alkaline hydrolysis is very effective at eliminating infectivity but is highly resource-intensive and lacks throughput. A typical alkaline hydrolysis unit can digest about 2 tons of biological material per cycle and relies on specific pH, temperature, and pressure conditions to inactivate prions (Murphy 2009). For context, adult white-tailed deer typically weigh 90 to 300 pounds, while adult elk range from 500 to 1,100 pounds (NPS 2024, USDA). After processing, the weight of the remains (e.g., bones, hide) can also vary widely, from about 40 pounds for an female adult white-tailed deer to several hundred pounds for a male adult elk (Field 2003, Ohio DNR).

Challenges and Barriers to Disposal Optimization

One perceived advantage of landfilling or burying cervid carcasses is that infectious CWD prions remain contained within that space. However, the fate of prions in landfill and soil matrices remains unclear, which raises concerns about the continued use of these methods. For example, little oversight exists to ensure proper burial of remains to avoid scavenger activity. Scavengers, especially birds, may transport prion-contaminated material to a new area, potentially introducing CWD to a naive cervid population (Fischer 2013). Furthermore, the endpoint of prion viability and migration within a landfill or upon burial in soil are also unknown. Results from one study that involved burial of BSE-infected material in soil suggested that infectivity was retained for at least 5 years and, although it remained largely confined to areas near the original source material, transport via rainwater may occur (Somerville 2019, Swire 2023). Thus, the hydrology of sites should be considered for onsite burial of CWD-infected carcasses, particularly if groundwater sources are nearby. It is also unclear if onsite burial of CWD-infected remains could permit future uptake of prions into plants growing in the affected area (Carlson 2023, Pritzkow 2015). While no studies tracking prion movement or infectivity in a real-world landfill environment have yet been published, results from an experiment that simulated various landfill conditions indicated that prion migration appears to be minimal, particularly when a layer of soil and/or liner separates infectious material from leachate-collection systems (Jacobson 2009). However, further research that involves larger-scale models, improved assays (e.g., SAAs, bioassays), and CWD-specific source material is needed to better characterize the fate of prions in landfills and determine what risk, if any, exists for CWD prion entry into leachate.

Another concern is that landfill operators' acceptance of potentially CWD-infected carcasses is waning as the disease becomes more widespread (Gillin 2018). Some facilities are owned by private companies, while others are owned by city municipalities, and their carcass disposal approaches vary. Some facilities do not accept carcasses at all, some require a negative CWD test for cervids, and some have no rules and do not require any testing (Anderson 2023). Acceptance is situational and difficult to predict, which complicates broad regulation of carcass and contaminated-item disposal.

Incineration and alkaline digestion disposal methods limit environmental contamination by destroying prion infectivity but currently lack the throughput and accessibility to address the large volume of waste generated from animal carcass disposal programs during hunting seasons. State and federal resources must be allocated to strengthen disposal capacity as a precaution against CWD spillover into a non-cervid production animal.

Furthermore, because no effective options exist for decontaminating environmental settings that harbor CWD prions, these unknowns raise questions about whether the considerable resources invested in carcass disposal are meaningful for disease management. For example, about 1.2 million deer die each year in the United States from motor vehicle collisions, another 6.1 million are harvested by hunting, and an unknown

number die from natural causes and are not accounted for (Anderson 2023). If even 1% of the estimated 7.3 million animals that die from hunting or collisions are infected with CWD, this represents 73,000 CWD-infected carcasses, many of which may not be disposed of using the methods outlined for CWD-infected cervids, and their remains decompose naturally in the environment. Thus, prion contamination of the environment related to carcass decomposition will continue and will likely increase with rising CWD prevalence over time, decreasing any potential benefit of formal carcass-disposal practices.

Current Disposal Regulation and Environmental Management for Animals with CWD and Other Prion Diseases

Cervids

A survey conducted in 2022 found that about three-quarters (38 of 50; 76%) of US state wildlife agencies had addressed cervid carcass disposal in their written CWD plans or disease response strategies (Anderson 2023). However, variability in disposal methods and guidance persists both within and between states, often due to differing regulatory frameworks, resource availability, and disease distribution. These inconsistencies can cause confusion among target audiences (e.g., hunters, taxidermists, meat processors) and result in limited awareness and support for applicable carcass-disposal programs.

Jurisdiction over disposal of farmed cervids typically falls on a state's department of agriculture or board of animal health (BAH). However, carcass-disposal regulations differ on a case-to-case basis. In Minnesota, for example, the BAH requires by law that all farmed cervids that die or are culled must be properly disposed of within 72 hours and explicitly prohibits leaving the carcasses on the open landscape (MN BAH). Producers of confirmed or suspected positive herds are offered several disposal options and instructed to work with the BAH to determine the best course of action. Anderson et al. interviewed professionals from the Minnesota BAH, who reported producers burying carcasses inside their fences, landfilling carcasses with cooperative facilities, composting indoors to prevent scavengers, and using multi-round alkaline digestion (Anderson 2023). These producer practices and compliance may differ from state to state.

In one Midwestern state, the Department of Natural Resources has asked the Department of Transportation, which is responsible only for major routes, to keep remains in the general area where they were found or as close as possible to that area (CIDRAP News, personal communication). Crews drag the animal to the back slope of the ditch in tall grasses or cover it with wood chips, mulch, or dirt if they are in a visible area. In urban areas where this may not be possible, the animals are taken to the closest rural area. These methods are followed strictly unless the area is mowed by an adjacent landowner or is by a driveway, in which case the animal is relocated to a nearby area.

Although some agencies have invested significant effort and resources into developing plans and practices for carcass disposal, the impact of these initiatives on CWD transmission in the environment remains largely unknown (Anderson 2023). Research deficits in this area may impede awareness about appropriate carcass disposal. Without evidence from the scientific community and agency willingness, regulations cannot be updated to align with the most current knowledge. Ideally, a CWD test result could be used to inform waste streams, but the people disposing of the materials often do not obtain results in time. In addition, the overall lack of uniform CWD testing within and between jurisdictions further limits the possible utility of this approach (Anderson 2023).

Non-Cervid Production Animals

The USDA conducts general BSE surveillance at slaughterhouses, where the agency

is required to test a minimum number of animals annually to fulfill surveillance requirements (USDA 2024). Products from these animals do not enter the food chain and are held for further testing. The BSE response plan suggests that the carcass may be disposed of before test results are available, if the animal is not intended for consumption.

According to USDA protocols, if a dead animal is suspected of having a neurological disease, the carcass (including the head, unless tested for rabies) is required to be held pending test results. If initial screening results are non-negative or positive, the USDA requests that Food Safety and Inspection Services personnel in the slaughter facility collect additional tissues for IHC and WB testing. When a clinically suspected BSE case occurs in Canada, carcasses are held until results are received. If BSE is confirmed or test results are non-negative, the carcass is sent to a reference lab for further necropsy.

The preferred methods of carcass disposal are alkaline digestion or incineration but, if the carcass is being held at a rendering facility, it could be buried in the interim and marked in case of exhumation. In situations in which a carcass needs to be exhumed for additional diagnostic testing, the remaining material should be disposed of using fixed-facility incineration or alkaline digestion.

Addressing Carcass Disposal in a Spillover Scenario Involving a Non-Cervid Production Animal

Unknown Variables of a Spillover Incident Related to Disposal

Although an incident has not yet been detected, CWD spillover to a non-cervid production animal would have considerable implications to disposal regulations. However, a number of unknown variables create challenges for generating authoritative policy on carcass and contaminated-item disposal. The USDA does not consider CWD a reportable disease in animals outside the cervid family, and thus resources such as the National Veterinary Stockpile's deployable incinerators may not be readily available. Without classification as a high-consequence foreign animal disease, the disposal aspects of a CWD spillover investigation may be slow to start.

Assuming the investigation proceeds without significant roadblocks, the animal species in which CWD spillover occurs will determine how disposal issues are handled. As evident in current cervid carcass regulations, resources are limited for using deactivation methods such as high-temperature incineration and alkaline digestion for whole carcasses. CWD spillover into cattle would require methods capable of handling a large amount of biological mass. Depending on the infected species, the new policy would also need to consider the added volume of biological waste generated by increased surveillance sampling and testing procedures.

At the time of detection, responders would not know the extent to which prions are distributed in the animal body. Peripheral spread outside of the CNS would implicate more tissues and body fluids as high-risk materials, requiring more careful consideration when veterinary services are sampling and transporting tissues from an infected animal. In addition, the spillover CWD prion strain may pose a greater threat to animals of the same species or humans.

Disposal Approaches Following Potential Spillover

In the context of a spillover to a non-cervid production animal, retaining access to the carcass for additional analysis will be critical, and disposal of any relevant tissues is not recommended. Analysis of non-CNS tissues may provide some insight into the distribution of CWD prions in non-cervid species. Prions in the lymphatic system and/or widely disseminated in the periphery would suggest potential horizontal transmission and thus imply facility contamination. The response plan would need to consider these factors.

Prion distribution assessment, however, takes time to assess experimentally.

After testing of the suspected index case and other potentially infected animals is complete, the approach to carcass disposal may shift to incineration or alkaline digestion to completely inactivate the prions, although accessibility and throughput will remain barriers. The number of animals affected would also influence the level at which these barriers impede proper disposal. A spillover incident will likely draw interest from agencies not typically associated with carcass disposal, such as public health and agriculture, which could also impact the decision-making process for carcass disposal.

It is unclear how disposal approaches for wild cervids would change following spillover into a non-cervid production animal. If confirmed, landfills that had historically disposed of hunter-harvested cervid carcasses may cease their agreements with wildlife agencies and other stakeholders. Political pressures will likely influence this regulation, and wildlife agencies will need to produce updated messaging to the public and hunters.

Key Findings

- CWD prions are highly stable in the environment and can remain infectious for years to decades, withstanding conditions that most other infectious agents cannot. With no effective way to inactivate prions in the environment at scale, the ongoing spread of disease presumably results in ever-increasing accumulation on the landscape.
- CWD prion fate and dynamics in the environment are difficult to characterize because of countless variables that span both the prion agent and the environment (e.g., climate, soil type, strain). Although some research has been conducted on this topic, the ability to conduct experiments in a controlled environment that mimics real-world conditions is limited.
- An effective, cost-efficient, and scalable disposal system for CWD-infected carcasses and contaminated items that limits environmental contamination has not been established. Each disposal method has notable drawbacks and limitations, and capacity is an issue for all options.
- Approaches to cervid carcass disposal vary across jurisdictions and agencies, as do the related information and communication about these approaches. These outcomes are shaped by factors such as regulatory authority and resource availability.
- Currently, CWD test results from hunter-harvested animals are often not delivered quickly enough to inform the most appropriate and timely disposal options. With an unknown CWD status, the safest approach may be to assume that animals are positive and dispose of them in ways that minimize risk.
- Information is lacking on the effect of carcass disposal measures on reducing the spread of CWD. Agencies rely on individuals to follow the recommended options, but compliance is largely unknown, and the proportion of CWD-positive animals being disposed of properly is unclear. A cost-benefit analysis is needed to assess the effectiveness of current carcass-disposal practices.
- CWD spillover into humans or non-cervid production animals would disrupt the disposal status quo and generate much higher volumes of waste to manage. The current system is not equipped to manage changes in demand.

References

Anderson C. Characterizing US Agency Approaches to Cervid Carcass Disposal in the Context of Chronic Wasting Disease Management: A Multi-State, Mixed-Methods Analysis. [Order No. 30571084]. University of Minnesota. 2023 [Full text]

Bartelt-Hunt SL, Bartz JC, Yuan Q. Prions in the Environment. In: Prions and Diseases. 2013 [Full document]

Bartz JC. Environmental and host factors that contribute to prion strain evolution. Acta Neuropathol. 2021 Apr 25;142(1):5-16 [Full text]

Baune C, Groveman BR, Hughson AG, et al. Efficacy of Wex-cide 128 disinfectant against multiple prion strains. PLoS One. 2023 Aug 24;18(8):e0290325 [Full text]

Brown P, Gajdusek DC. Survival of scrapie virus after 3 year's interment. Lancet. 1991;337:269-70 [Full text]

Carlson CM, Thomas S, Keating MW, et al. Plants as vectors for environmental prion transmission. iScience. 2023 Nov 10;26(12):108428 [Full text]

Davies P, Brown DR. Manganese enhances prion protein survival in model soils and increases prion infectivity to cells. PLoS One. 2009 Oct 21;4(10):e7518 [Full text]

Field RA, Smith FC, Hepworth WG, et al. The elk carcass. Agricultural Experiment Station, B-594R. University of Wyoming. 2003 Aug [Full document]

Fischer JW, Phillips GE, Nichols TA, et al. Could avian scavengers translocate infectious prions to disease-free areas initiating new foci of chronic wasting disease? Prion. 2013;7(4): 263-66. [Full text]

Georgsson G, Sigurdarson S, Brown P. Infectious agent of sheep scrapie may persist in the environment for at least 16 years. J Gen Virol. 2006 Dec;87(Pt 12):3737-40 [Full text]

Gillin C, Mawdsley J, eds. AFWA Technical Report on Best Management Practices for Prevention, Surveillance, and Management of Chronic Wasting Disease. Association of Fish and Wildlife Agencies. 2018 [Full document]

Henderson DM, Davenport KA, Haley NJ, et al. Quantitative assessment of prion infectivity in tissues and body fluids by real-time quaking-induced conversion. J Gen Virol. 2015 Jan;96(Pt 1):210-9 [Full text]

Holec SAM, Yuan Q, Bartz JC. Alteration of prion strain emergence by nonhost factors. mSphere 2019 Oct 9;4(5):e00630-19 [Full text]

Johnson CJ, Pedersen JA, Chappell RJ, et al. Oral transmissibility of prion disease is enhanced by binding to soil particles. PLoS Pathog. 2007 Jul 6;3(7):e93 [Full text]

Johnson CJ, Phillips KE, Schramm PT, et al. Prions adhere to soil minerals and remain infectious. PLoS Pathog. 2006 Apr;2(4):e32 [Full text]

Jacobson KH, Lee S, McKenzie D, et al. Transport of the pathogenic prion protein through landfill materials. Environ Sci Technol. 2009 Mar;43(6): 2022-8. [Full text]

Kuznetsova A, McKenzie D, Ytrehus B, et al. Movement of chronic wasting disease prions in prairie, boreal and Alpine soils. Pathogens. 2023 Feb 7;12(2):269 [Full text]

Maddison BC, Owen JP, Bishop K, et al. The interaction of ruminant PrP(Sc) with soils is influenced by prion source and soil type. Environ Sci Technol. 2010 Nov 15;44(22):8503-8 [Full text]

Minnesota Board of Animal Health. Farmed cervid carcass disposal [Web page]

Murphy RG, Scanga JA, Powers BE, et al. Alkaline hydrolysis of mouse-adapted scrapie for inactivation and disposal of prion-positive material. J Anim Sci. 2009 May;87(5):1787-93. [Full text]

National Park Service. White-tailed deer (Odocoileus virginianus). 2024 [Web page]

Ohio Department of Natural Resources. Venison meat yield [Full document]

Pritzkow S, Gorski D, Ramirez F, et al. Prion dissemination through the environment and medical practices: Facts and risks for human health. Clin Microbiol Rev. 2021 Dec 15;34(4):e0005919 [Full text]

Pritzkow S, Morales R, Moda F, et al. Grass plants bind, retain, uptake and transport infectious prions. Cell Rep. 2015 May 14;11(8):1168-75 [Full text]

Saunders SE, Bartelt-Hunt SL, Bartz JC. Prions in the environment: Occurrence, fate and mitigation. Prion. 2008 Oct-Dec;2(4):162-9 [Full text]

Saunders SE, Bartz JC, Bartelt-Hunt SL. Prion protein adsorption to soil in a competitive matrix is slow and reduced. Environ Sci Technol. 2009 Jul 15;43(14):5242-8 [Full text]

Saunders SE, Shikiya RA, Langenfeld K, et al. Replication efficiency of soil-bound prions varies with soil type. J Virol. 2011 Jun;85(11):5476-82 [Full text]

Somerville RA, Fernie K, Smith A, et al. BSE infectivity survives burial for five years with only limited spread. Arch Virol. 2019 Feb 24;164(4):1135-45 [Full text]

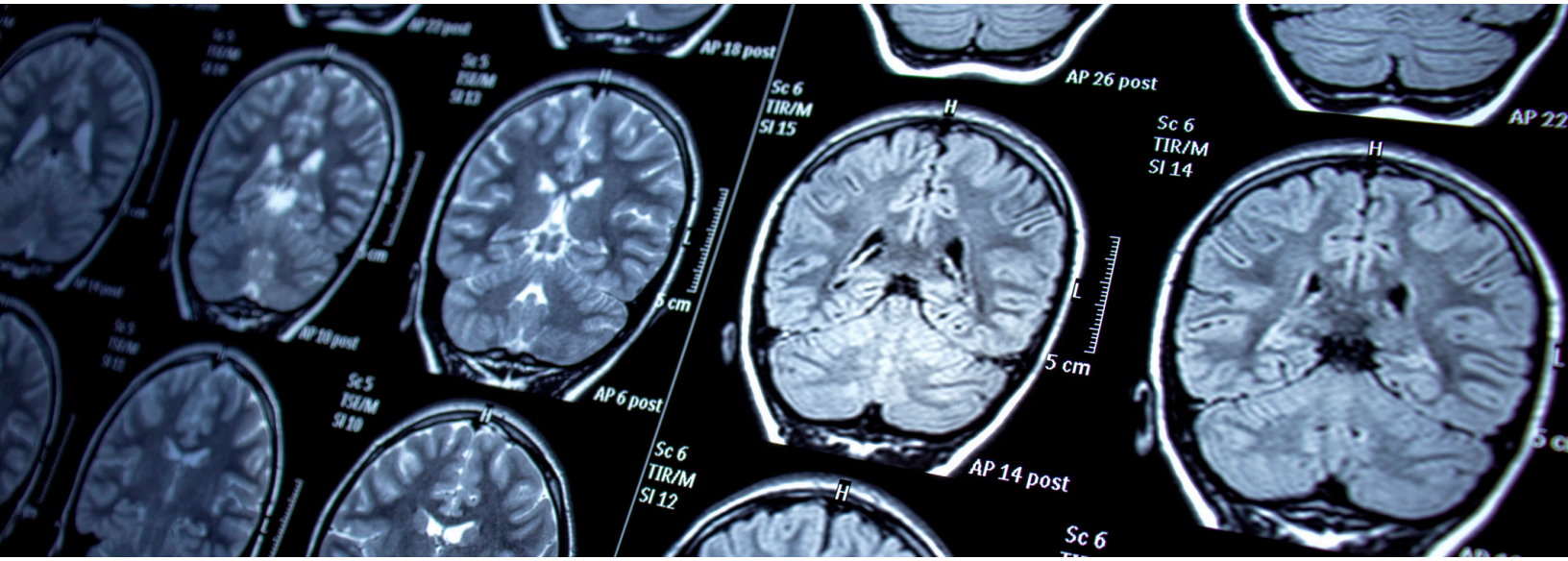
Swire E, Colchester A. Out of sight, out of mind? BSE 30 years on: continuing environmental risks to human health. Land Use Policy. 2023 Mar;126. [Full text]

US Department of Agriculture. BSE Surveillance Information Center. 2024 [Web page]

US Department of Agriculture. Chronic Wasting Disease Program Standards. 2019 [Full document]

US Department of Agriculture. Elk [Web page]

Yuan Q, Eckland T, Telling G, et al. Mitigation of prion infectivity and conversion capacity by a simulated natural process—Repeated cycles of drying and wetting. PLoS Pathog. 2015 Feb 9;11(2):e1004638 [Full text]



Chapter 5: Detecting CWD Spillover into Humans

Introduction

Although the public has generally viewed CWD as an animal health issue, ongoing spread of the disease in cervids has led to growing concerns about its potential to spill over into humans. Given the potential consequences of such a scenario and considering the precedent set with BSE and vCJD, CWD warrants the ongoing attention of public health officials to ensure that activities and plans are in place to detect and respond to potential human spillover events. This chapter examines existing diagnostics and surveillance systems for human prion disease and outlines current challenges with responding to a spillover.

History of Zoonotic Prion Disease in Humans

Classical BSE, resulting from the consumption of contaminated feed, serves as a model for zoonotic prion transmission to humans. This form of BSE was first identified as a disease affecting cattle herds in the United Kingdom in 1986 and was epidemiologically linked to supplemental feeding practices that fed prion-contaminated feed containing meat-and-bone meal to calves (Collee 1997). The original source of prion contamination remains unknown but is hypothesized to be either of bovine origin (through spontaneous misfolding of cattle proteins and subsequent amplification via the supplemental feed) or ovine origin (scrapie prions that contaminated ruminant feed crossing the species barrier to infect cattle). The BSE outbreak prompted changes to supplemental feeding regulations and practices in cattle, including a ban on using ruminant protein for ruminant feeds (1988); a ban on using brain, spinal cord, and other specified bovine offals in feed for nonruminant animals and poultry (1990); and restrictions on processing cattle older than 30 months (1996) (Beale 2001). Over many years, these regulations effectively eliminated BSE among these herds after proper enforcement and biosafety measures were implemented (Schonberger 1998). Because scrapie, another prion disease first described in sheep in 1732 (Zabel 2015), had never been documented as causing disease in humans and was regarded as the probable source of the BSE outbreak, some United Kingdom officials assured the public that products

from affected animals were safe to consume. However, in 1996 (10 years after BSE was initially recognized in cattle), UK surveillance systems initiated to monitor BSE spillover concerns began identifying unusual cases of neurodegenerative disease in adults under 45 years old, considerably younger than typical CJD patients (Will 1996). An expert advisory committee concluded that the most likely explanation for the newly classified cases of vCJD was a causal link to prior consumption of BSE-contaminated food products, based on their analysis of the unique epidemiological, clinical, neuropathological, and protein chemistry features of these cases (Beck 2005, Houston 2019).

The unique neuropathology of vCJD cases and the results of large-scale mortality data analyses provided evidence that the identification of this atypical prion disease in young adults (median age 29 years in 10 cases first described by Will et al.) was not simply the result of the UK's national surveillance program initiation (Will 1996). As of 2024, 233 cases of vCJD had been identified, with 178 in the United Kingdom and 55 reported from 11 other countries (Diack 2014, Ritchie 2021). Of the 55 cases originating outside of the United Kingdom, most had either resided in the United Kingdom when BSE exposure was high or resided in a non-UK country that had imported presumably contaminated food products, including meat-and-bone meal feed, from the United Kingdom during the UK BSE outbreak (Ritchie 2021). Recognition of the vCJD outbreak occurring 10 years after the BSE epidemic in cattle illustrates the potentially long incubation period of zoonotic prion diseases; if CWD transmits to humans, it may be years or even decades before the outbreak becomes clinically apparent.

Since the vCJD outbreak, the research community has worked to characterize the mechanism of transmission from cattle to humans. Prion pathogenesis relies primarily on ingestion of contaminated materials (Sigurdson 2018); thus, animal models using oral exposure to infectious prions are most illustrative of a natural course of infection in a human. Several studies have demonstrated that orally exposing primates to different doses of BSE prions (PrP^{BSE}) results in BSE disease transmission with a high attack rate, and as is characteristic of prion diseases in general, always leads to death (Holznagel 2013).

Human Prion Disease Diagnostics

Antemortem Testing and Limitations

Recognizing prion diseases in humans can be challenging, particularly antemortem. According to the CDC's latest diagnostic criteria for CJD, postmortem testing is needed for definitive diagnosis, unless an adequate brain biopsy is performed (CDC 2024b, CWRU 2024c, Figgie 2021). However, owing to the invasive nature and infection-control issues of brain biopsies, these procedures are typically not recommended unless it is a suspected treatable illness. Recent advancements in diagnostic methodologies and tools have enhanced the utility of less-invasive antemortem testing (Figgie 2021). In particular, RT-QuIC, which can be performed on cerebrospinal fluid (CSF) or certain other tissues, has revolutionized antemortem diagnosis of the most common types of prion diseases in the United States (Hermann 2022, Ng 2024). For example, a recent study co-authored by investigators at the National Prion Disease Pathology Surveillance Center (NPDPSC) and the CDC reported that RT-QuIC testing of CSF had a sensitivity of 90.3% and a specificity of 98.5% for diagnosing cases of any prion disease in the United States that are referred to the NPDPSC and verified by tissue analysis (Rhoads 2020). Thus, its application is valuable, especially when used in combination with other diagnostic tests, such as brain magnetic resonance imaging (MRI) (Appleby 2022, CWRU 2024b, Figgie 2021).

Despite these improvements, important limitations to antemortem testing remain, including the following:

- No single test is completely accurate across all types and subtypes of prion disease. For example, depending on the subtype, the sensitivity of certain tests can be noticeably reduced. Even with RT-QuIC, certain subtypes appear to produce more false-negative results. Research has shown that samples of brain tissue homogenate and CSF from vCJD cases result in inefficient RT-QuIC reactions (Peden 2012). This variability raises concerns about potential diagnostic challenges that may arise if CWD spills over to humans, because the sensitivities of available tests in such a scenario are unknown (Figgie 2021).
- Prion diseases are heterogeneous, and symptoms often overlap with other neurologic conditions; therefore, a consultation from a neurologist or clinician with experience diagnosing neurological conditions may be important for clinically recognizing the disease. Because prion diseases are relatively rare, most clinicians have limited experience with them, which can hamper timely and accurate diagnosis.
- Adequate access to healthcare, particularly specialty care, is crucial for antemortem diagnosis of prion diseases. Ultimately, healthcare access (or lack thereof) can shape test availability, especially in rural areas, where populations are more likely to hunt (USFWS 2022). Furthermore, the effectiveness of certain tests (e.g., brain MRI) is clinician-dependent, and results are most beneficial when assessed by specialists with expertise in prion disease diagnosis.

Postmortem Testing

Each year in the United States, about one or two cases of diagnosed prion disease occur per 1 million people (330 to 660 cases per year). Most of these cases are sporadic CJD (sCJD), which has a lifetime risk of around 1 in 5,000 (Jones 2020). In 2023, the NPDPSC received 327 neuropathology referrals and, of those, 237 (72.4%) were positively identified as a prion disease, with a large majority (216 of 237; 91.1%) attributed to sCJD (CDC 2024b, CWRU 2024d). A definitive and specific diagnosis of prion disease is possible only through neuropathologic examination, which is almost exclusively conducted via autopsy (Appleby 2022, Figgie 2021, Zerr 2024). This process can be initiated through a number of different channels but ultimately involves obtaining and analyzing brain tissue samples (ideally, the entire brain) from patients with suspected prion disease. In the United States, clinical samples undergo testing at the NPDPSC, which was established by the CDC in 1997 in response to the UK vCJD outbreak and is located at Case Western Reserve University in Cleveland, Ohio. Continued CDC funding has enabled the NPDPSC to coordinate the free collection and testing of tissue samples for US clinicians, families affected by prion disease, and public health officials. Prion disease experts at the NPDPSC use WB, histopathologic testing, IHC, and genetic analysis in addition to review of clinical history and known or potentially acquired prion disease risk factors (NPDPSC Testing Form). WB characterizes prion proteins extracted from tissue samples, histopathology examines the tissue structure microscopically, IHC visualizes prions microscopically in a tissue sample via antibodies, and genetic testing examines the *PRNP* genotype to identify prion disease risk factors. Tested cases and their specimens may also contribute to prion disease-related special studies such as: (1) the evaluation of the sensitivity and specificity of various clinical tests to diagnose different types of prion disease; (2) international genetic studies to identify genes that affect susceptibility to prion disease; and (3) experimental transmission studies in transgenic mice.

WB is primarily used to examine frozen brain tissue, while freshly harvested and fixed tissue is needed for histopathology and IHC analyses. Thus, the diagnostic approach depends on the condition of the tissue samples that arrive at

the NPDPSC. Both tissue types are preferred, because histopathology, IHC, and WB generate different insights about a case. IHC not only detects prions but also characterizes their localization and aggregation patterns across diverse brain tissue types. Of importance, these tests are complementary to identify the specific prion strain present in brain tissue.

Another critical component of the diagnostic process at the NPDPSC is sequencing of the decedent's *PRNP* gene (i.e., the gene that encodes normal cellular prion proteins) (CWRU 2024a). This approach is primarily used to identify pathogenic protein forms, indicating a genetic origin of the prion disease. However, even with non-hereditary disease, sequencing still has value because genetic predisposition to human prion diseases is well established, with certain polymorphisms associated with increased susceptibility and the potential to influence key disease characteristics (Appleby 2022). For example, virtually all people diagnosed with vCJD with genetic information available (160 of 161, 99.4%) have been methionine homozygous (MM) at codon 129 in the *PRNP* gene (NCJDRSU 2022, Ritchie 2021).

Overall, each of these tests provides important information on specific prion disease characteristics and, when used in combination, can ultimately help distinguish prion subtypes. For example, each of the six known subtypes of sCJD has a unique constellation of features, including, but not limited to, prion deposition in certain parts of the brain, distinct molecular properties, and particular polymorphisms at codon 129, as well as clinical and diagnostic differences (Appleby 2018, Belay 2003, Figgie 2021). With these tests, different characteristics can be identified, and the collective results can reveal the associated subtype (Holper 2022). Furthermore, by using a combination of histopathology, IHC, or WB testing and genetic sequencing, atypical cases of prion disease (e.g., vCJD, possible human CWD spillover) can be recognized, which reinforces the value and necessity of postmortem examination (Figgie 2021, Louie 2004).

Despite the critical role of postmortem testing in prion disease diagnosis, several challenges remain:

- Autopsy rates as a whole and for prion disease diagnosis in particular have declined since 2019, which limits the ability to confirm prion diseases and identify new, atypical cases. Reasons for this decline are likely complex and situation-dependent but may include the availability of improved antemortem testing methods, perceived cost of autopsies (despite these services being paid for by the CDC through the NPDPSC in the United States), concerns about infection control, lack of clinician concern about the patient having had a novel prion disease, and time elapsed since the vCJD outbreak. Regardless, a reduction in the number of autopsies lowers the potential for timely recognition of a CWD spillover or provision of evidence-based reassurance should unfounded public fears develop concerning the emergence of human CWD.
- Both the availability of tissues for postmortem examination and their quality can vary, ultimately affecting the analyses that can be performed. For example, postmortem tissue biopsies do not provide the same level of diagnostic clarity as the entire brain. In addition, access to extraneural tissues is generally limited, despite the potential value of examining such tissues in understanding human prion diseases and identifying a possible case of human CWD.
- Researchers do not know how CWD would present in humans. Thus, uncertainties exist regarding how best to identify a case and whether cases would be recognized through current clinical practice, particularly given the absence of a laboratory test (pre- or post-mortem)

that can determine whether CWD caused a human prion disease.

Human Prion Disease Surveillance

Prion Disease Surveillance in North America

In public health, infectious disease surveillance is a tool for understanding morbidity and mortality due to specific infections at a population level. Ideally, obtaining data through an established surveillance system is the best way to monitor disease occurrence. Such monitoring offers valuable insights into disease dynamics and can inform strategies for disease prevention and control. Currently, several systems work in parallel to monitor human prion diseases in the United States, including:

- Follow-up by public health agencies of reports received from healthcare personnel, laboratories, or non-medical sources such as family members.
- Periodic reviews of the Multiple Cause Of Death Data from state vital statistics departments that are routinely submitted to the CDC's National Center for Health Statistics.
- Funding and support of the NPDPSC to provide free, state-of-the-art prion disease neuropathology diagnostic services to US physicians.
- Pursuing more focused, systematic, and long-term monitoring of prion diseases in high-risk subpopulations. These groups include recipients of tissue or human growth hormone donations from CJD patients, or more recently, cervid hunters and consumers of venison in states in which CWD is well established.

Human prion diseases, namely CJD, are not nationally notifiable in the United States, but most states participate in some form of CJD surveillance, albeit with varied reporting criteria. For example, Minnesota has deemed all patients with clinically suspected and confirmed prion disease, regardless of age, reportable within 1 business day, while Georgia requires reporting of only patients younger than 55 within 7 days. Clinicians or others who report cases to local and state public health authorities are strongly encouraged to contact the NPDPSC to coordinate an autopsy (MDH 2024) or provide information to families about free autopsy availability so they may contact the NPDPSC. The NPDPSC provides no-cost coordination and autopsy services to analyze harvested tissue samples and diagnose disease postmortem. The NPDPSC also performs RT-QuIC analysis on CSF samples from living patients and provides results, although not free of charge (Rhoads 2020). Once processed, the results are reported back to the local provider, the CDC, and state health departments. Currently, the NPDPSC is the only nationally dedicated US center that provides conclusive testing services and critical support to national surveillance on human prion diseases, accepting a range of autopsy inquiries to optimize case capture.

Annual US case numbers of prion disease, updated quarterly, are available on the NPDPSC website (CWRU 2024d). The CDC merges its death certificate data with NPDPSC-confirmed cases, as well as a few reports it receives directly, usually from clinicians and health departments (Holman 2010). Because prion diseases are relatively rare and invariably fatal, about 80% of prion disease cases identified through active surveillance methods can be identified through death certificates alone, and the use of this combination of previously described case ascertainment methods should more accurately capture national prion disease incidence (Holman 2010).

In addition, the CDC supports eight states in conducting enhanced CJD surveillance (i.e., active plus passive), including Texas and California (Texas DSHS, CEIP) plus special epidemiologic studies among hunters in Colorado, Wisconsin, and Wyoming. This work

focuses on suspected cases of prion disease in people younger than 55 and features questions about patient history of harvesting and consuming a CWD-positive cervid. The CDC is working to expand these efforts, primarily in states with high CWD prevalence. In addition, with the family's permission, lymph tissue is collected from decedents at high risk for exposure to CWD whose remains are evaluated through the NPDPSC-facilitated autopsy program. If a suspected CWD spillover case is found, this measure will help determine if the prion agent is in lymph tissue, making it more likely to be found in blood.

Prion disease surveillance in Canada is centrally operated through the federal Public Health Agency of Canada (PHAC) (Canada PHA 2024). All human prion diseases are provincially reportable and nationally notifiable, with testing conducted at the National Microbiology Laboratory. For CNS testing, a modified QuIC test is used that has a sensitivity of 95% and specificity of 99% for diagnosing verified cases of human prion disease (Simon 2020). Surveillance includes follow up for each suspected case by phone interview to collect clinical and epidemiological information using a standardized questionnaire. Autopsies are organized and funded by the PHAC, and the acceptance rate of autopsies among suspected cases is more than 60%.

Current Prion Disease Surveillance Challenges

Although systems are in place, several limiting factors to current surveillance hinder the ability to assess the full picture of human prion disease in the United States. For example, physician education and recognition of atypical prion disease are deficient. The general presentation of prion disease includes rapid-onset dementia and a range of non-specific neurological symptoms, which may be mistaken for other conditions. If the attending physician never suspects or orders tests for prion disease, the case remains undiscovered. Neurologists may be more likely to recognize prion disease signs, but many communities across the country lack access to specialized medicine.

Even if prion disease is suspected, opportunities to investigate atypical cases via brain tissue analysis are limited if the patient dies before diagnosis and an autopsy has not been coordinated. The UK surveillance model requires in-person clinical follow-up whenever possible for every suspected case of prion disease, a thorough practice that played a large role in case identification during the vCJD crisis. However, this approach is resource intensive and largely unsustainable in the United States. The NPDPSC recently began a teleneurology assessment program for CJD that aims to address this gap in diagnostics and surveillance (CWRU 2024e); however, these standardized clinical evaluations and histories are only performed in less than 10% of all expected prion disease cases.

The NPDPSC can gather data only from the autopsy referrals that the center coordinates and performs, which is about half the expected number of prion disease cases in the country. In 2022, the NPDPSC received just 337 referrals for neuropathologic examination (CWRU 2024d). The NPDPSC has the capacity to perform a higher number of procedures but is limited by the referrals it receives. This deficit may be attributed to challenges with convincing a patient's next of kin to donate the decedent's brain, despite these services being free of charge. Because of the growing accessibility of SAA technology, other labs across the country have begun performing RT-QuIC, decentralizing the CDC's approach to surveillance in which the NPDPSC has been the only US prion disease diagnostic lab providing RT-QuIC testing of human CSF samples. Currently, no official collaboration agreements allow results to be shared with the NPDPSC, so other labs may not report positive test results in a timely manner or at all, decreasing the CDC's ability to detect prion diseases. It is also important for physicians to understand that RT-QuIC is not sufficient for

a complete diagnosis and differentiation of prion disease type.

Limitations to one of CDC's current surveillance mechanisms include a lag in processing death certificates and a reliance on unverified cause-of-death information that may have been given before the patient's final diagnosis. Complete mortality data submitted to the CDC can be several years behind, delaying the analysis and incorporation of death certificate data into surveillance reports. Such delays constitute another reason that CDC supports the NPDPSC and relies on the center's data and prompt reporting for more timely and specific prion disease surveillance information. An unusually high occurrence or clustering of CJD in young people, along with an exposure to CWD-infected cervids, would certainly raise alarm about potential transmission of CWD to humans.

Surveillance system sensitivity relies on factors such as the likelihood of healthcare providers to consider the diagnosis and order appropriate testing, the ability of diagnostic testing to accurately diagnose the condition, the capacity of public health agencies to investigate suspected prion disease cases, and the likelihood that current surveillance activities will capture the case (CDC 2001). US surveillance for prion diseases is limited because healthcare providers may not always consider the diagnosis, and appropriate tissues may not be available for centralized testing and reporting through the NPDPSC. Thus, current US surveillance for prion disease may not be sensitive enough to rapidly identify early CWD spillover to humans. Therefore, it is critical to address barriers to implementing more robust surveillance measures so that particular prion disease spillover indicators do not go undetected.

Owing to the long latent period associated with prion diseases and the potential for delayed identification of a CWD spillover to humans, the CDC recommends that, despite the theoretical nature of human CWD risk, people (particularly cervid hunters) avoid or minimize potential contact with CWD (CDC 2024a). The CDC also offers specific guidelines to hunters on how to reduce their risk of exposure to infectious CWD prions.

Investigation of a Suspected CWD Spillover Event to Humans

Implications

A CWD spillover event in humans in the United States or Canada is most likely to be detected through a combination of an epidemiological assessment of multiple unusual cases and neuropathological evaluations of brain tissue at a prion disease surveillance laboratory. Until a laboratory test becomes available to identify human CWD, single spillover cases are unlikely to be confidently identified.

Another way a human CWD spillover event may be discovered is through cohort studies in which participants have a high likelihood of exposure to infectious prions, such as the ongoing CDC monitoring of hunters in Colorado, Wyoming, and Wisconsin in collaboration with health departments in the affected states. The Oneida County Chronic Wasting Disease Surveillance Project, also an example of such studies, was started in 2005 in response to a known exposure in a group of 81 people who consumed CWD-contaminated venison at a sportsmen's feast (Olszowy 2014). The research group is continuing follow-up with the cohort but has not detected any cases of prion disease to date (Ralph Garruto, personal communication). If any cases of prion disease occur in this or other cohorts, even if such cases resemble sCJD, investigators will likely suspect CWD transmission and consider the implications. Some researchers have argued that the lack of evidence to support cross-species transmission of CWD makes transmission to humans unlikely. However, two issues must be considered when assessing future risk. First, the incubation period for CWD in humans is likely to be long (i.e., 10 years or more), so the clinical landscape will always lag behind the real-time transmission risk. Second, while the

attack rate of humans exposed to CWD prions in a hypothetical spillover event is unknown, experience from the UK vCJD outbreak suggests that it may be low: of roughly 5 million UK residents estimated to have consumed BSE-infected beef, only 178 (3.5 per 100,000) primary vCJD cases were identified (Chen 2014). A low attack rate may necessitate studies with very large cohort sizes to detect spillover transmission of CWD. Third, risk of CWD spillover cannot be regarded as static because of increasing wildlife prevalence, frequency and dose of prion exposure, and strain characteristics, which could facilitate cross-species transmission, may change over time. Finally, different CWD strains present with different experimental host ranges and incubation periods, and some strains may pose a higher zoonotic transmissibility risk. Thus, past experience does not necessarily predict current or future risk.

As with a CWD spillover in a production animal, the response to a suspected CWD case in a human will require both epidemiologic and laboratory investigations. The combined priorities of these investigations include determining the origin of infection, assessing the ongoing risk to humans, and characterizing transmission pathways and disease manifestation in affected humans.

Laboratory Investigation

Because no documented cases of CWD-linked clinical disease have been identified in humans, researchers do not know how a spillover case would present clinically and if it would be recognized. Furthermore, no formal diagnostic protocol is able to classify a case of prion disease as CWD-related. In the United States, assuming the affected patient is deceased and appropriate tissues from autopsy are forwarded to the NPDPSC, neuropathologists there will examine samples via histopathology, IHC, WB, and *PRNP* genotyping, as discussed in "Postmortem Testing," above. Unlike a non-cervid animal spillover, in which reference materials from earlier experimental inoculation studies may be available for tissue comparison, no existing tissue samples will be available from humans. Instead, results using strains of interest from experiments with cell lines, humanized mice, or SAAs using human tissue will be available, but these resources may be sparse and their interpretation challenging (Thapa 2022, Sun 2023). Therefore, CWD spillover neuropathology in humans will likely be uncharacterized and may initially lead to misclassification as an atypical form of human prion disease or sCJD. A priority for the laboratory investigation will be to determine that the disease is not classified as sCJD and that it represents a distinct pathology linked to CWD prion protein (PrP^{CWD}) exposure and infection. If CWD spillover is confirmed, specific strain typing will be useful in identifying additional cases, although the technology requires additional resources and work for optimization.

Epidemiologic Investigation

In a suspected case of CWD spillover, an epidemiologic investigation will be initiated to determine exposure status and risk factors to identify any other cases. Questions about CWD are included on the standard NPDPSC autopsy consent form and CDC CJD case report form, although more detail would build evidence of a spillover. Consideration of infectious prion dose and period of exposure would give investigators a better picture of how the index case was infected and whether other populations may be at risk. However, retrospective data collection may be limited because patients with suspected cases could have been exposed multiple times at unknown doses, frequency, or duration. These data may be subject to recall bias if subjects are asked about lifetime exposure, which is difficult to remember accurately and completely. If the patient has already died, family members may provide information that is likely to be biased as well.

More prospective studies that compare the incidence of prion disease in groups of hunters who have consumed large amounts of venison versus non-hunters who have consumed relatively little may be helpful. In

addition, non-hunters may consume more venison in some situations than the hunters themselves (e.g., family members of hunters). Studies may be subject to bias or confounding, so it will be important to use appropriate study designs and analytic methods to best assess CWD exposure as a potential cause of human prion disease.

If an above-expected number of cases with a similar, unique pathology can be identified, a case definition will be developed to describe pertinent features of the index CWD spillover case and standardize the identification of additional cases. This neuropathological grouping was used early in the UK BSE crisis as an alternative to looking for geographic or temporal clustering (Will 1996). This approach may be particularly useful for CWD, because geographic clustering may not occur, given that CWD in cervids is widespread in the United States and Canada, and many people consume venison.

Investigative Challenges

In the past, CJD case clusters prompted suspicion of CWD spillover to humans. In those instances, individuals were linked geographically, temporally, or socially through participation in common hunting activities (Rong 2023, Trout 2024). However, state health department, NPDPSC, and CDC investigators jointly concluded that cervid-to-human transmission was not the cause of CJD in these patients and that their connections occurred by chance (White 2024). Regardless of this reassurance, reactions from the public tend to be mixed when supposed clusters are covered in the media (Snider 2024). This attention may present investigative challenges if a true CWD spillover incident were to occur.

Both confirming and ruling out possible CWD transmission to humans is laborious and will likely be characterized by uncertainty. First, no definitive test can unquestionably demonstrate etiology in a timely manner; therefore, officials will not know the source of the index infection at the time the investigation begins. This greatly hinders investigators' ability to establish criteria to define human exposure to a spillover event. Although historical evidence from the BSE epidemic suggests that consumption of CWD-contaminated tissue would be the most likely source of infection, this assumption overlooks environmental exposure routes (e.g., food contaminated via contact with contaminated plants, dust, or processing equipment) and percutaneous routes of infection such as cuts during field dressing. Current detection methods may also be limited in their ability to confirm the absence of CWD prions in a sample, as discussed in Chapter 2, particularly because prion disease in human CWD spillover cases may involve unusual locations in the brain or elsewhere in the body. Non-CNS involvement was observed in cases of vCJD where prions were found to accumulate in lymphoreticular tissue (Hilton 2004). Thus, testing of brain tissue only could limit the ability of neuropathologists to assess non-CNS involvement in suspected cases. Furthermore, as CWD prevalence increases and expands, the distinction between exposed and unexposed populations becomes less clear, particularly as more research findings emerge about the extended persistence of CWD prions in the environment.

Very few efficient and specific testing approaches can identify the spillover origin. Prion diseases are different from other infectious diseases because an infectious prion is essentially the same protein that the human body normally produces but with a unique folding pattern. The chemical identity of two types of infectious prion protein from the same host (i.e., comparing samples from a CWD spillover case to a historical vCJD case) makes characterizing novel origin and strain properties difficult. Aside from bioassays, which would demonstrate a strain's ability to infect a species in vivo, PMCA could compare a strain's ability to infect different species and tissue types more rapidly in vitro (Harpaz 2023). However, these assays will likely be insufficient to establish case exposures or differentiate the cause as consumption

of contaminated animal products versus environmental exposure.

In a spillover scenario, it may be important to involve more than one external research lab and establish a priority system beforehand to determine who can obtain a sample from the spillover case (or cases) and for what reason. This may require organizations to work through logistical hurdles such as drafting policy and standard operating procedures, which may be an extended, complicated process. Such policies, if enacted, could establish a critical research network to identify future CWD spillover cases.-

One Health Agency Collaboration

Combining agency efforts through comprehensive disease management strategies is likely the best way to decrease the risk and spread of CWD, but viewing CWD exclusively as an animal health issue and dismissing potential zoonotic transmission may stifle public health agency involvement. Cross-sectional collaboration with wildlife scientists and veterinarians, public health practitioners, and human health experts in a One Health approach is essential to creating an effective and long-lasting disease-management plan (Gilch 2022).

Iatrogenic vCJD and Prion Disease Infection Control

Iatrogenic CJD

Cases of iatrogenic CJD (iCJD) from exposures in healthcare settings have been linked to medical procedures, particularly in recipients of contaminated human growth hormone and dura mater (Brown 2012). iCJD cases identified in the 1980s can be traced back to procedures that took place before strict donor screening practices and recordkeeping were instituted and before synthetic hormone was available. Since these changes, the incidence of iCJD has decreased significantly, but cases continue to occur because of the prolonged incubation periods of prion diseases.

After the UK BSE crisis, many countries began implementing systematic leukodepletion, a practice that greatly reduced the concentration of white blood cells, shown to be associated with prion infectivity in blood (Brown 2001, Lacroux 2012). This practice was instituted because of concerns about vCJD transmission via the blood supply and was effective in preventing transfusion-related iatrogenic vCJD spread. An extensive surveillance of UK vCJD cases related to blood transfusion identified five probable cases who may have acquired clinical vCJD or misfolded proteins after receiving a blood or plasma-derived product from a donor who later developed clinical vCJD. All of these patients received a non-leukodepleted blood-derived product in the United Kingdom from 1994 to 1999 (di Borgo 2023). The Red Cross led a large, long-term vCJD response program that led to the exclusion of UK blood donors and had far-reaching impacts (Dietz 2007, Crowder 2017, Seed 2018). Although secondary transmission of infectious vCJD prions through blood transfusion has been documented, the current risk of CJD transfusion transmission is theoretical, according to findings from a study conducted by the CDC and American Red Cross (Crowder 2017). Many countries are now reassessing biosecurity regulations surrounding this issue, especially after the adoption of leukodepletion practices for donated blood further reduced risk.

With vCJD, there was also concern about the serial use of tools in surgical procedures that had undergone sterilization procedures that did not necessarily inactivate prion infectivity (Stevenson 2020). For a time, this uncertainty prompted a shift to using single-use instruments to eliminate risk (Richards 2007). vCJD was detected in patient peripheral tissues, but transmission of vCJD through surgical procedures has not been documented (Head 2004).

Biosafety Measures After Potential CWD Spillover

If transmission of CWD from its natural host to a human is confirmed, researchers do not know whether the associated transfusion-transmission risk would be the same as that observed in human-to-human vCJD transmission (Mammadova 2020, di Borgo 2023). As an emerging infectious disease, human CWD would require significant consideration of existing policies surrounding donor-acceptance guidelines and the sterilization of surgical tools. Guidelines and resources from the UK BSE outbreak may be crucial in shaping early strategies for a CWD spillover incident.

Public health authority to investigate possible routes of transmission, such as human-to-human transmission of human CWD via blood, tissue, or organ donation, may be limited by current federal or state regulations and policies. Research organizations may be allowed to assist with further risk assessment; however, public health agencies may require an emergency declaration to investigate these routes of potential transmission.

Key Findings

- The risk of CWD spillover is not static, and factors such as rising disease prevalence in wildlife and evolving CWD strain characteristics may alter transmissibility to non-cervid species over time. Historical data, therefore, cannot be considered a reliable predictor of current or future spillover risk.
- The zoonotic risk of CWD is not understood in the context of growing exposure, prolonged incubation periods that often accompany interspecies transmission of CWD, or evolving CWD prion strains. Although experimental transmission studies are valuable and inform the understanding of zoonotic risk, extrapolating results and applying them to the real world is difficult because of numerous limitations.
- Physician recognition of prion diseases is limited because these illnesses are rare and have symptoms that overlap with other neurodegenerative diseases. Also, important barriers remain to antemortem testing and diagnosis. Prion disease manifestation in humans can vary by age, prion strain, and other factors that could influence recognition of disease, particularly for patients without access to specialized care.
- Although autopsies are important for investigating and diagnosing prion disease, the number of autopsies completed for this or any other purpose is decreasing, likely due to numerous factors. Coupled with the increasing prevalence of CWD among cervids and the risk of exposure to CWD among humans, the power of the current surveillance system to detect a single case of CWD spillover is limited.
- CWD transmission to a human is uncharacterized, and researchers have not defined the evidence (clinical, pathological, or epidemiologic) that would identify a spillover incident. Identification would likely depend on the recognition of multiple cases with similar disease features and presumed CWD exposure.
- Obtaining exposure information relevant to prion diseases is challenging, particularly given potentially prolonged incubation periods. Furthermore, widespread gaps in cervid CWD surveillance, outdated consumption estimates for CWD-positive cervids, and extensive but undocumented exposure (i.e., non-hunter venison consumers and environmental exposure) limit the collection of these data.
- CWD spillover-related collaboration among public health, wildlife, and agriculture agencies is somewhat limited. Obstacles include cooperation/agency buy-in, issues related to data privacy (e.g., wildlife agencies sharing hunting license data with public health), funding, and the significant amount of unknowns on the subject.
- Because suspicion of a CWD spillover incident may be met with resistance, skepticism, and fear, pre-scripted messaging and consensus recommendations would be useful in addressing inquiries.

References

Appleby BS, Rhoads DD, Mente K, et al. A practical primer on prion pathology. J Neuropathol Exp Neurol. 2018 May 1;77(5):346-52 [Full text]

Appleby BS, Shetty S, Elkasaby M. Genetic aspects of human prion diseases. Front Neurol. 2022 Oct 5;13:1003056 [Full text]

Beale AJ. BSE and vCJD: What is the future? J R Soc Med. 2001 May;94(5):207-9 [Full text]

Beck M, Asenova D, Dickson G. Public administration, science, and risk assessment: A case study of the U.K. bovine spongiform encephalopathy crisis. Pub Admin Rev 2005 Jul/Aug;65(4):396-408 [Full text]

Belay ED, Maddox RA, Gambetti P, et al. Monitoring the occurrence of emerging forms of Creutzfeldt-Jakob disease in the United States. Neurology. 2003 Jan 28;60(2):176-81 [Full text]

Brown P, Brandel JP, Sato T, et al. Iatrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis. 2012 Jun;18(6):901-7 [Full text]

Brown P, Cervenáková L, Diringer H. Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease. J Lab Clin Med. 2001 Jan;137(1):5-13 [Full text]

CDC. About Chronic Wasting Disease (CWD). 2024 April 14 [Web page]

CDC. CJD Diagnostic Criteria. 2024 May 13 [Web page]

CDC. Classic Creutzfeldt-Jakob Disease. 2024 May 13. [Web page]

CDC. Updated guidelines for evaluating public health surveillance systems, Recommendations from the Guidelines Working Group. MMWR 2001 Jul 27;50(RR13)1-35 [Web page]

California Emerging Infections Program. Creutzfeldt-Jakob Disease (CJD) [Web page]

Case Western Reserve University. Autopsy Testing Reporting. 2024 [Web page]

Case Western Reserve University. Brain Magnetic Resonance Imaging (MRI). 2024 [Web page]

Case Western Reserve University. Diagnostic Criteria for Creutzfeldt-Jakob Disease (CJD). 2024. [Web page]

Case Western Reserve University. NPDPSC Table of Cases Examined. 2024. [Web page]

Case Western Reserve University. Teleneurology Assessment Program for Creutzfeldt-Jakob Disease. 2024. [Web page]

Chen CC, Wang YH. Estimation of the exposure of the UK population to the bovine spongiform encephalopathy agent through dietary intake during the period 1980 to 1996. PLoS One. 2014 Apr;9(4) [Full text]

Collee JG, Bradley R. BSE: A decade on--Part I. Lancet. 1997 Mar 1;349(9052):636-41 [Full text]

Crowder LA, Schonberger LB, Dodd RY, et al. Creutzfeldt-Jakob disease lookback study: 21 years of surveillance for transfusion transmission risk. Transfusion. 2017 Aug;57(8):1875-8 [Full text]

di Borgo AP, Rochette S, Gaussen A, et al. Transmission of variant Creutzfeldt-Jakob disease through blood transfusion and plasma-derived products: A narrative review of observed and modeled risks. Transfus Med Rev. 2023 Jul;37(3):150747 [Full text]

Diack AB, Head MW, McCutcheon S, et al. Variant CJD: 18 years of research and surveillance. Prion. 2014;8(4):286-95 [Full text]

Dietz K, Raddatz G, Wallis J, et al. Blood transfusion and spread of variant Creutzfeldt-Jakob disease. Emerg Infect Dis. 2007 Jan;13(1):89-96 [Full text]

Figgie MP Jr, Appleby BS. Clinical use of improved diagnostic testing for detection of prion disease. Viruses. 2021 Apr 28;13(5):789 [Full text]

Gilch S. Chronic wasting disease—A prion disease through a One Health lens. Can Vet J. 2022 Apr;63(4):431-3 [Full text]

Harpaz E, Vuong TT, Tran L, et al. Inter- and intra-species conversion efficacies of Norwegian prion isolates estimated by serial protein misfolding cyclic amplification. Vet Res. 2023 Sep 29;54(1):84 [Full text]

Head MW, Ritchie D, Smith N, et al. Peripheral tissue involvement in sporadic, iatrogenic, and variant Creutzfeldt-Jakob disease: An immunohistochemical, quantitative, and biochemical study. Am J Pathol. 2004 Jan;164(1):143-53 [Full text]

Hermann P, Appleby B, Brandel JP, et al. Biomarkers and diagnostic guidelines for sporadic Creutzfeldt-Jakob disease. Lancet Neurol. 2021 Mar;20(3):235-46 [Full text]

Hilton DA, Sutak J, Smith ME, et al. Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. J Clin Pathol. 2004 Mar;57(3):300-2 [Full text]

Holman RC, Belay ED, Christensen KY, et al. Human prion diseases in the United States. PLoS One. 2010 Jan 1;5(1):e8521 [Full text]

Holper S, Lewis V, Wesselingh R, et al. Comprehensive clinical, radiological, pathological and biochemical analysis required to differentiate VV1 sporadic Creutzfeldt-Jakob disease from suspected variant CJD. BMJ Neurol Open. 2022 Apr 18;4(1):e000299 [Full text]

Holznagel E, Yutzy B, Schulz-Schaeffer W, et al. Foodborne transmission of bovine spongiform encephalopathy to nonhuman primates. *Emerg Infect Dis*. 2013;19(5):712-720 [Full text]

Houston F, Andréoletti O. Animal prion diseases: The risks to human health. Brain Pathol. 2019 Mar;29(2):248-62 [Full text]

Jones E, Hummerich H, Viré E, et al. Identification of novel risk loci and causal insights for sporadic Creutzfeldt-Jakob disease: a genome-wide association study. Lancet Neurol. 2020 Oct;19(10):840-48 [Full text]

Lacroux C, Bougard D, Litaise C, et al. Impact of leucocyte depletion and prion reduction filters on TSE blood borne transmission. PLoS One. 2012;7(7):e42019 [Full text]

Louie JK, Gavali SS, Belay ED, et al. Barriers to Creutzfeldt-Jakob disease autopsies, California. Emerg Infect Dis. 2004 Sep;10(9):1677-80 [Full text]

Mammadova N, Cassmann E, Greenlee JJ. Successful transmission of the chronic wasting disease (CWD) agent to white-tailed deer by intravenous blood transfusion. Res Vet Sci. 2020 Dec;133:304-6 [Full text]

Minnesota Department of Health. Health Professionals: Creutzfeldt-Jakob Disease (CJD). 2024 May 21. [Web page]

The National CJD Research & Surveillance Unit (NCJDRSU). 31st Annual Report 2022: Creutzfeldt-Jakob Disease Surveillance in the UK. [Full text]

Ng D, Watson N, McDermott EA, et al. Characterisation of RT-QuIC negative cases from the UK National CJD Research and Surveillance programme. J Neurol. 2024 Jul;271(7):4216-26 [Full text]

National Prion Disease Pathology Surveillance Center. National Prion Disease Pathology Surveillance Center Testing and Reporting Policies. [Full document]

Olszowy KM, Lavelle J, Rachfal K, et al. 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. 2014 Sep;128(9):860-8 [Full text]

Peden AH, McGuire LI, Appleford NEJ, et al. Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. J Gen Virol. 2012 Feb;93(2):438-449 [Full text]

Public Health Agency of Canada. Creutzfeldt-Jakob Disease Surveillance System. 2024 Sep 10 [Web page]

Richards D. Decontamination, single-use instruments and vCJD. Evid Based Dent. 2007;8(2):32-3 [Full text]

Ritchie DL, Peden AH, Barria MA. Variant CJD: Reflections a quarter of a century on. Pathogens. 2021 Oct 30;10(11):1413 [Full text]

Rhoads DD, Wrona A, Foutz A, et al. Diagnosis of prion diseases by RT-QuIC results in improved surveillance. Neurology. 2020 Aug 25;95(8):e1017-e1026 [Full text]

Rong LL, Lannen NJ, Tank EC, et al. Case report: Two clusters of Creutzfeldt-Jakob disease cases within 1 year in West Michigan. Front Neurol. 2023 Mar 20;14:1134225 [Full text]

Schonberger LB. New variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. Infect Dis Clin North Am. 1998 Mar;12(1):111-21 [Full text]

Seed CR, Hewitt PE, Dodd RY, et al. Creutzfeldt-Jakob disease and blood transfusion safety. Vox Sang. 2018 Apr;113(3):220-231 [Full text]

Sigurdson CJ, Bartz JC, Glatzel M. Cellular and molecular mechanisms of prion disease. Annu Rev Pathol. 2018 Oct 24;14:497-516 [Full text]

Simon SLR, Peterson A, Phillipson C, et al. Prospective study demonstrates utility of EP-QuIC in Creutzfeldt-Jakob disease diagnoses. Can J Neurol Sci. 2021 Jan;48(1):127-129 [Full text]

Snider M. CDC: Deer meat didn't cause hunters' deaths; Concerns about chronic wasting disease remain. USA Today. 2024 Apr 26. [Full article]

Stevenson M, Uttley L, Oakley JE, et al. Interventions to reduce the risk of surgically transmitted Creutzfeldt–Jakob disease: A cost-effective modelling review. In: Health Technology Assessment, No. 24.11. 2020 Feb. [Full text]

Sun JL, Telling GC. New developments in prion disease research using genetically modified mouse models. Cell Tissue Res. 2023 Apr;392(1):33-46 [Full text]

Texas Department of State Health Services. Creutzfeldt-Jakob Disease (CJD) [Web page]

Thapa S, Marrero Winkens C, Tahir W, et al. Gene-edited cell models to study chronic wasting disease. Viruses. 2022;14(3):609 [Full text]

Trout J, Roberts M, Tabert M, et al. Two hunters from the same lodge afflicted with sporadic CJD: Is chronic wasting disease to blame? Neurology. 2024 Apr 9;102(Suppl. S1):P7-13.002 [Abstract]

US Fish & Wildlife Services. 2022 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation. 2023 Sep [Full document]

White R. Michigan woman dies from rare brain disease that's 100% fatal. She's not the first. M Live. 2024 Apr 2 [Full article]

Will RG, Zeidler M. Diagnosing Creutzfeldt-Jakob disease. BMJ. 1996 Oct 5;313:833-4 [Full text]

Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet. 1996 Apr 6;347(9006):921-5 [Full text]

Zabel MD, Reid C. A brief history of prions. Pathog Dis. 2015 Dec;73(9):ftv087 [Full text]

Zerr I, Ladogana A, Mead S, et al. Creutzfeldt-Jakob disease and other prion diseases. Nat Rev Dis Primers. 2024 Feb 29;10(1):14 [Full text]

Conclusions and Recommendations

The following recommendations are the result of extensive discussions among experts in 25 working group meetings spanning five topic areas (wildlife management, agriculture, human medicine, prion research, and contaminated materials disposal). They highlight and prioritize gaps and needed actions to prepare for the possibility of CWD spillover into other wildlife, non-cervid production animals, or humans.

1. Advocate for CWD spillover awareness and preparedness among medical providers, research institutions, and wildlife, agricultural, and public health agencies.
2. Create sustained, dedicated, multi-year funding sources to support needed efforts.
3. Expand and standardize CWD surveillance in wild cervids, other wildlife, and non-cervid production animals to facilitate data summarization and interjurisdictional comparisons.
4. Promote human prion disease surveillance among human healthcare providers to enhance capacities to detect CWD spillover to humans.
5. Expand CWD research and development efforts.
6. Encourage and support formalized interagency and cross-sector collaboration.
7. Develop and test proactive CWD spillover communications, messaging, and education.
8. Draft regulatory and government agency policies in advance.
9. Plan for environmental concerns and estimate disposal capacity needs.

1. Advocate for CWD spillover awareness and preparedness among medical providers, research institutions, and wildlife, agricultural, and public health agencies.

There is a risk of CWD spillover to other wildlife, non-cervid production animals, and humans. The risk of CWD spillover is not static and may be increasing as new strains emerge and exposure probabilities increase. Historical data may not be a reliable predictor of current or future spillover risk. As a result, there are growing needs for:

- Additional capacity and infrastructure to support CWD research, diagnostic innovation, and preparedness;
- New communication strategies to inform policymakers and stakeholders who would be responsible for a successful CWD spillover identification and response; and,
- Advanced planning, in particular multi-agency tabletop exercises to prepare consistent and coordinated CWD spillover policies and response plans.

2. Create sustained, dedicated, multi-year funding sources to support needed efforts.

Currently available funding is insufficient and largely available only in single-year grants that are poorly designed to meet the concerns raised in this report. As a result, there are increasingly urgent needs to:

- Diversify funding support for state and tribal wildlife agencies so that they can effectively address new interdisciplinary conservation challenges, of which CWD surveillance and response are only one. Hunting license fees, linked excise taxes, and available USDA funding are grossly insufficient and would be incapable of supporting an effective CWD spillover response. Furthermore, if spillover to humans occurs, deer hunting license sales likely will significantly decrease, compounding budget gaps.
- Build laboratory capacity and develop less expensive, faster, and more sensitive CWD testing to address increases in testing demand following a spillover incident.
- Prioritize research funding (See below: "Expand CWD research and development efforts") to improve early detection and diagnosis of CWD spillover. This should include robust support for basic, hypothesis-driven research that advances scientific understanding and innovation.
- Bolster funding for state, tribal, and federal public health agencies to ensure timely and effective CWD spillover response.
- Educate public health officials and healthcare professionals to improve their understanding of and surveillance for prion diseases.

3. Expand and standardize CWD surveillance in wild cervids, other wildlife, and non-cervid production animals to facilitate data summarization and interjurisdictional comparisons.

Expanded and improved surveillance for CWD is needed in both cervids and targeted non-cervid species to improve the likelihood of detecting potential spillover incidents. Focusing on clinical, pathologic, and epidemiologic evidence will improve surveillance, inform understanding of disease dynamics, and guide public health responses.

- Conduct an assessment of the financial and logistical requirements necessary to improve lab-based surveillance capabilities on a state-by-state basis.
- Systematically expand laboratory capacity to increase sample throughput and decrease testing costs and times so that laboratories can accommodate surges in

CWD testing demand (e.g., during hunting seasons).

- Incorporate strain-level data into existing cervid CWD surveillance programs to improve understanding of disease dynamics and management needs.
- Expand and standardize CWD surveillance programs to monitor disease prevalence in both wild cervids and other wildlife species, including targeted non-cervid species that may share habitats with production animals.
- Integrate data from research that tests the natural environment for CWD contamination to optimize agency surveillance protocols.
- Establish provisional criteria for surveillance protocols and identification of CWD spillover incidents among non-cervid wildlife and production animals.

4. Continue and promote human prion disease surveillance, review and refine public health guidance, and educate healthcare providers to enhance capacities to detect CWD spillover.

- Expand the number of longitudinal cohort studies that prospectively follow groups that face greater exposure likelihoods, especially people who frequently consume hunter-harvested deer or elk meat from areas with high CWD prevalence.
- Strengthen coordination between public health agencies, the National Prion Disease Pathology Surveillance Center (NPDPSC), and other laboratories that conduct prion testing of clinical samples from patients with neurodegenerative disease to ensure centralization of US surveillance for human prion disease.
- Develop strategies to promote the consistent collection of detailed and standardized patient histories and risk factor information for human prion disease cases.
- Continue to raise awareness of the importance of autopsy on suspected cases of human prion disease.
- Formalize public health guidance for scenarios with potential opportunities for exposure to the CWD agent, including testing and basic personal protective equipment recommendations (e.g., wearing gloves when field-dressing a deer carcass).
- Enhance physician awareness of (1) human prion disease, with a focus on the potential risk of CWD spillover from consumption of CWD-contaminated meat, particularly in areas with high CWD prevalence in cervids, (2) location-specific reporting requirements of prion disease cases, and (3) the diagnostic services that NPDPSC provides to confirm human prion disease in suspected cases.
- Obtain updated population statistics on hunters and dietary habits regarding CWD risk factors.

5. Expand CWD research and development efforts.

There is a pressing need to better understand evolving CWD prion strain characteristics and associated spillover risk.

- Conduct additional large-animal studies to assess the risk of interspecies transmission and document clinical signs, tissue distribution, and shedding potential of CWD prions in non-cervid hosts infected via the oral-nasal route. Additional facilities capable of housing large animals for research are necessary to fill this gap.
- Develop and integrate accessible strain-typing technology into CWD surveillance protocols to investigate strain properties as a contributing factor to transmissibility and virulence.

- Support the innovation and delivery of new rapid (and antemortem) diagnostic tests that can identify CWD in cervids and non-cervid species and support surge testing needs.
- Accelerate the development and validation of rapid (and antemortem) diagnostic tests.
- Refine understanding of the dynamics and infectivity of CWD prions in environmental media (e.g., soil, water, vegetation) through optimized research methods.
- Identify factors that influence CWD prion persistence in environmental media and test potential mitigation strategies.
- Improve understanding of interspecies and environmental transmission risks.
- Support and expand the tissue repository under development at the National Wildlife Research Center.
- Expand human dimensions studies related to CWD.

6. Encourage and support formalized interagency and cross-sector collaboration.

Proactive collaboration among agencies that regulate wildlife, public health, agriculture, and the environment is needed to develop integrated CWD management strategies, including those that address spillover risks.

- Formalize and foster relationships across local, state, regional, tribal, and federal agencies to facilitate CWD coordination, management, and response.
- Collaborate to develop interagency CWD spillover contingency plans that can be included in state CWD response plans, clarify roles and responsibilities, and organize communication and response efforts.
- Foster interdisciplinary collaborations among government agencies, researchers, public health officials, wildlife managers, tribal nations, veterinarians, and physicians to facilitate sharing of knowledge, data, resources, and best practices related to prion disease research, CWD surveillance, and spillover risk and response.
- Obtain required laboratory permits in advance to enable non-governmental (e.g., academic) experts to contribute to spillover investigations.
- Establish a scientific advisory group of prion biology, neurology, CWD, and policy experts to support and advise government agency investigations of apparent CWD spillover incidents, including providing assessments of and recommended responses to the associated public health risk as needed.

7. Develop and test proactive CWD spillover communications, messaging, and education.

Government agencies should consider preparing messaging and language in advance to address various levels of public health risk posed by a CWD spillover incident under investigation. Different approaches may be needed for different audiences.

- Align messaging efforts with other emerging-disease communications, focusing on the facts to help maintain trust early in a CWD spillover investigation.
- Develop separate educational messaging for targeted professionals (e.g., healthcare providers, veterinarians, wildlife personnel) about prion diseases in general, and CWD specifically, to improve understanding and recognition of prion diseases and potential CWD spillover cases. This may highlight clinical signs that overlap between identified spillover cases and other

neurodegenerative diseases of a respective species.

- Develop pre-scripted messaging and consensus recommendations for the general public to address anticipated public inquiries about CWD spillover risks, anticipating skepticism and fear and fostering trust through transparent communication.
- Identify innovative methods to engage different audiences with spillover communications to ensure the receipt of information, including those with "disease fatigue" and different generations that may obtain information differently.
- Consider initiatives to counteract CWD-related misinformation on various platforms.

8. Draft regulatory and government agency policies in advance.

To the extent possible, policies and data use agreements should be drafted proactively, periodically reviewed and updated, and available should a spillover occur.

- Develop general recommendations for a spillover testing protocol for federally regulated diagnostic laboratories. Specific recommendations may vary by species or location.
- Continue to pursue validation of prion seeding amplification assays to facilitate their acceptance and use in diagnostic investigations.
- Draft policies that prioritize ecosystem health and data-driven actions for potential spillover response by state wildlife agencies, including guidance for targeted cervid surveillance and any anticipated changes to hunting activities. These policies should be developed in collaboration with the National Fish and Wildlife Health Initiative steering committee, which includes personnel from state and federal wildlife agencies, the USDA, and tribal nations.
- Prepare and expedite data use agreements or memoranda of understanding so data may be shared between different agencies and, possibly, other stakeholders while investigating a potential CWD spillover incident.
- Continue to promote the use of electronic tagging systems in all facilities housing captive cervids and across animal agriculture to improve animal traceability and aid in potential spillover investigations.

9. Plan for environmental concerns and estimate disposal capacity needs.

Assessments of the impact of disposal activities on the environmental burden of CWD and disease outcomes, as well as disposal capacity needs, are necessary to adapt and refine disposal strategies over time should a spillover occur.

- Establish long-term monitoring programs to assess the environmental impact of current carcass disposal practices and CWD prion dynamics.
- Allocate state and federal resources to strengthen capacity for disposal of CWD-infected carcasses and other contaminated materials as a precaution for CWD spillover into non-cervid production animals. Groups of neighboring states could work together to address disposal needs and share large facilities and associated costs.
- Standardize protocols for carcass and contaminated-item disposal across jurisdictions to ensure consistency and efficiency, considering local environmental conditions and prion stability.
- Develop innovative, cost-effective technologies for disposal of CWD-infected

cervid and non-cervid production animal carcasses.

- Comprehensive cost-benefit analyses of current carcass disposal methods to evaluate their effectiveness in preventing CWD spread are also needed to inform policy changes and resource allocation.

Appendix A: CWD Surveillance and Testing Capacities of State Agencies

J.R. Mason, PhD

Justification

Chronic wasting disease (CWD) is spreading steadily across North America, despite preventive regulations and other mitigations (e.g., sharpshooting). As a result, CWD exposures among humans and livestock probably are increasing.

The available evidence suggests that there are substantial barriers to CWD spillover from cervids to other species. Nevertheless, it's reasonable to speculate that the probability of a spillover event could be growing as a function of exposures, because they provide opportunities for new (and perhaps more susceptible) combinations of individual genes and CWD strains.

Should a spillover event occur, demand for more disease surveillance probably would increase. This, in turn, would increase the number of samples submitted to diagnostic laboratories for testing. Whether the existing national capacity for CWD testing could accommodate substantial increases in testing is an open question.

Methods

We explored state agency perspectives on surveillance and testing capacities through a brief survey that the Association of Fish and Wildlife Agencies (AFWA) distributed to state agency wildlife chiefs in Spring 2024.

We asked four questions:

1. How many CWD samples per year does your state test now?
2. Who do you send samples to for analysis?
3. If demand surged to ≥10,000 CWD samples/year, could your state accommodate the additional testing?
4. If CWD testing demand exceeded your state's existing capacity, does your agency have an agreement with additional laboratories who could assist in meeting demand?

Results and Discussion

Eventually, 27 surveys were returned. Of these, 19 were returned from states where CWD had been identified in free-ranging cervids. When sorted by AFWA regional associations, 11 were returned by Western Association of Fish and Wildlife Agencies (WAFWA) states, 10 came from Southeastern Association of Fish and Wildlife Agencies (SEAFWA) states, 8 were returned by Midwestern Association of Fish and Wildlife Agencies (MAFWA) members, and 3 came from Northeastern Association of Fish and Wildlife Agencies (NEAFWA) states.

Overall, the number of CWD tests conducted per state and year ranged from 0 to 36,146, with a mean ± standard error of the mean (SEM) of 7,208 ± 1,791. Among CWD-positive (CWD+) states,

the number of samples tested per state ranged from 199 to 36,146, with a mean and SEM of 9,707 ± 2,318. Among CWD-negative (CWD-) states, the number of samples tested per state ranged from 0 to 3,250, with a mean and SEM of 1068 ± 373.

The ranges, means (± SEM) for samples tested by CWD+ and CWD- states within each of the four regional AFWA were:

- WAFWA
  - CWD+ states: Range, 199 to 24,500; 6,403 ± 2646
  - CWD- states: Range, 15 to 3,250; 1,063 ± 529
- SEAFWA
  - CWD+ states: Range, 199 to 36,146; 13,818 ± 4,759
  - CWD- states: Range, 0 to 1,500; 750 ± 536
- MAFWA
  - CWD+ states: Range, 600 to 30,000; 11,139 ± 3,622
  - CWD- states: There are no CWD- states in the MAFWA.
- NEAFWA
  - CWD+ states: Only one NEAFWA CWD+ state responded. In that state, an average of 1,500 samples are tested annually.
  - CWD- states: Only 2 NEAFWA CWD- states responded. In those states, the average numbers of samples tested annually were 860 and 2,700, respectively. The mean sampling rate was 1,780.

When asked where samples were sent for CWD testing, 25 states indicated that samples were shipped to a National Animal Health Laboratory Network (NAHLN) veterinary diagnostic laboratory. NAHLN facilities in Colorado and Wisconsin were used a little more than half the time (52%). Outside the NAHLN, two states sent samples to the Southeastern Wildlife Disease Cooperative Study laboratory, one sent samples to the University of Pennsylvania Wildlife Futures Laboratory, and one state indicated that samples were tested "in-house."[1]

When asked if they had the capacity to handle a surge of at least 10,000 samples, states typically answered that they had, or could reallocate funding to achieve, surveillance activities capable of collecting at least 10,000 samples. Conversely, just four states indicated that they had, or could reallocate funding to achieve, a testing capacity to analyze at least 10,000 samples. Ten states indicated that they might be able to accommodate a surge 6,000 or more samples, while five reported that they would not be able to accommodate a surge. One state indicated that it would refuse to accommodate more samples than they were testing now, citing biosecurity concerns. In that instance, previous high testing volumes (~30,000 samples/year) led or contributed to several laboratory personnel becoming infected with bovine tuberculosis (an endemic disease of white-tailed deer in a portion of that state).

No state reported that they had formal arrangements for surge testing with testing facilities besides the facility already being used. One state reported that it would definitely be able to establish an arrangement with an additional laboratory, 20 reported that they might be able to do so, and one did not think that it would be able to establish a secondary laboratory.

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1 The number of sample testing laboratories exceeds the number of states that submitted surveys because one state submitted samples to more than one laboratory (e.g., for confirmation of an initial positive finding).

Appendix B: Glossary

Alimentary tract: The digestive tract, which includes the mouth, throat, esophagus, stomach, intestines, rectum, and anus.

Alkaline hydrolysis: A method of disposing of human and animal remains with heat, water, and lye rather than burial or cremation.

Attack rate: The proportion of people who become ill with or die from a disease in a population initially free of the disease.

Baiting: The use of deer feeding and attractants to draw cervids to a specific area for hunting purposes.

Bioassay: A test that measures the effect of a substance on a living organism to determine its concentration.

Bovine: A group of medium to large animals such as cattle, bison, buffalo, and antelope.

Bovine spongiform encephalopathy (BSE): A fatal prion brain disease also known as "mad cow disease" that occurs in cattle and has been transmitted to humans.

Atypical BSE: Form of BSE that occurs spontaneously at very low levels in all cattle populations, particularly cattle 8 years of age or older, and does not appear to be associated with contaminated feed. To date, there is no evidence that atypical BSE is transmissible.

Classical BSE: Form of BSE that results from the ingestion of cattle feed containing the abnormal prion protein and has been linked to variant Creutzfeldt-Jakob disease (vCJD) in humans.

Cervids: Hoofed ruminant animals such as deer, moose, elk, and caribou.

Chromosome: Genetic material made up of a protein and a DNA molecule inside the nucleus of animal and plant cells.

Chronic wasting disease (CWD): A fatal neurodegenerative disease of cervids such as white-tailed deer, mule deer, elk, moose, and reindeer caused by infectious proteins called prions.

Codon: A sequence of three basic structural units of DNA or RNA that instruct cells to start, add to, or stop protein chains.

Creutzfeldt-Jakob disease (CJD): A fatal degenerative human brain disease caused by prions and characterized by impaired memory, behavioral changes, visual problems, dementia, and hallucinations.

Cryogenic electron microscopy: A method that uses a transmission electron microscope to determine the 3D structure of biomolecules.

Empiric: Based on observation or experience alone.

Endemic: Prevalent in a particular area or environment.

Epidemiology: The branch of medical science that studies factors that determine the presence or absence of diseases to learn how many people are affected, if that number is changing, and how the disease is affecting society and the economy.

Enzyme-linked immunosorbent assay (ELISA): A common laboratory test used to detect antibodies to a pathogen in blood samples.

Epitope: The portion of an antigen to which antibodies attach.

Etiology: Source or origin.

Genetic sequencing: A laboratory process that generates genetic information.

Genotype: Complete set of genetic material in an organism.

Geomembrane: A synthetic, waterproof membrane that controls the exchange of fluids or gases.

Homogenate: A tissue preparation made by breaking down the tissue structure and cells.

Homozygous: Having two identical versions of a gene, with one inherited from each parent.

Horizontal transmission: The process of transmitting organisms, directly or indirectly, from one host to another.

Iatrogenic: Related to a disease caused by medical treatment or examination.

Immunohistochemistry (IHC): A laboratory method that uses antibodies to detect and visualize disease markers in tissue samples.

Immunoreactivity: The response of a substance to an antigen or protein.

Incubation period: The time it takes to develop symptoms after exposure to an infectious disease.

Inoculum: A part of an infectious pathogen used for inoculation, or immunization.

In vitro: Performed in a controlled laboratory environment such as a petri dish or test tube.

In vivo: Within a whole, living organism such as an animal, human, or plant.

Isoform: Functionally similar protein with a similar, but not the same, amino acid sequence.

Kuru: A rare fatal neurodegenerative human disease caused by prions that leads to tremors and lack of coordination.

Leachate: Water that has percolated through a solid mass, leaching out some components.

Leukodepletion: A process by which leukocytes, or white blood cells, are removed from donated blood to, in the case of CWD, reduce prion infectivity.

L-type bovine spongiform encephalopathy (BSE): A rare, atypical form of BSE that occurs spontaneously in cattle, especially in older animals.

Methionine: An essential amino acid that has an important role in health and metabolism.

Necropsy: Surgical examination of a dead animal to determine the cause of death or scope of disease, a process akin to autopsy in humans.

Obex: Region of the brain at which the fourth ventricle narrows into the spinal cord's central canal.

One Health: An approach that recognizes the interconnectedness of the health of people, animals, and the environment.

Optical density: A logarithmic quantification of light passing through a material.

Passage: The transfer or subculture of cells from one vessel to another.

Pathogenesis: The process by which a disease develops and advances.

Pathognomonic: Specifically characteristic or indicative of a particular disease or condition.

Peripheral lymph nodes: Organs in the lymphatic system that filter lymph and have an important role in immune response.

Polymorphism: The presence of two or more variant forms of a DNA sequence in individuals or populations.

Prion: Infectious protein that can trigger normal proteins in the brain to fold abnormally.

Production animal: A farmed animal raised for products such as meat, eggs, milk, wool, leather, or labor.

Proteinase K: An enzyme that breaks down proteins. Resistance to this enzyme is used in laboratory tests to differentiate between normal host proteins and infectious prions.

***PRNP*:** Human prion protein gene designation

***Prnp*:** Non-human prion protein gene designation

PrP^{BSE}: Abnormal prion protein that aggregates in the brain of bovine spongiform encephalopathy (BSE)-infected animals.

PrP^{C}: Normal cellular prion protein.

PrP^{CWD}: Abnormal prion protein that aggregates in the brain of CWD-infected cervids.

PrP^{Sc}: Abnormal, disease-associated form of the prion protein.

Radio-frequency identification tag: A type of small electronic tag that uses radio waves to communicate data to a reader.

Rectoanal mucosa-associated lymph tissue (RAMALT): The part of the lymphoid system that can be used to diagnose transmissible spongiform encephalopathies such as CWD.

Retropharyngeal lymph nodes: Lymph nodes found in the retropharyngeal space, which lies between the base of the skull and the C3 vertebra (near the base of the neck).

Scrapie: A fatal prion brain disorder that occurs in sheep and goats.

Sensitivity: The ability of a test to identify infected animals or humans with a specific disease.

Specificity: The ability of a test to identify animals or humans without a specific disease.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE): A method used to separate proteins in a mixture based on size.

Seed amplification assay (SAA): A test developed for the detection of prion proteins. It is also known as real-time quaking-induced conversion (RT-QuIC) or protein misfolding cyclic amplification (PMCA).

Species barrier: The natural barrier assumed to prevent diseases from spreading from one species of animal or plant to another.

Spillover: Event in which a pathogen moves from one species into a new host, causing infection and a potential outbreak.

Strain: Distinct strains of prions exist and are operationally defined as a heritable phenotype of disease under controlled agent and host conditions.

Substrate: The material on which an organism lives or derives nourishment.

Subtitle D landfill: A solid-waste landfill that cannot allow hazardous waste.

Sympatric: Occurring in the same geographic area.

Titer: Concentration of a substance in a solution.

Transgenic: Related to an animal that contains DNA from an unrelated animal for research.

Tropism: Growth or movement in response to an external stimulus.

Transmissible spongiform encephalopathies (TSEs): Rare degenerative brain disorders in humans and other animals caused by prions and characterized by tiny holes that give the brain a spongy appearance.

Usufructuary: A person who has the right to use another person's property.

Western blot (WB): A laboratory test that identifies specific proteins in a tissue or blood sample.

Zoonotic: Caused by pathogens transmitted between animals and humans.