

# Tuesday, May 30<sup>th</sup>, 2023

## Rocky Mountain National Park Tour 7:00 AM- 6:00 PM

7:00am - 4:00pm Tuesday, 30th May, 2023  
Sheraton Lobby

Pre-Registration Required

Join Dr. Jenny Powers, Wildlife Health Branch Chief at the National Park Service, for a day trip discovering the majestic beauty of the Rocky Mountains, the cervids that inhabit the park, and the threat they face from CWD.

Please meet promptly at 7:00 am (location)

## ***Registration 4:00 pm - 6:00pm***

## **Welcome Reception**

5:00 - 6:00pm Tuesday, 30th May, 2023  
Plaza Ballroom

Cash bar available

## **CWD Perspectives Panel Discussion**

6:00 - 7:30pm Tuesday, 30th May, 2023  
Plaza Ballroom

CWD has an impact on many different groups. In this panel representatives from Tribes, State Departments of Natural Resources, the farmed cervid industry, and Departments of Agriculture will give their perspectives on the importance of cervids to their respective groups and the impact of CWD. There will be some prepared questions and an opportunity for audience members to ask questions of the panel. The goal is to provide a respectful forum to increase understanding between interest groups.

### **Panelists:**

Joe Hagberg, CWD Coordinator, Blackfeet Fish & Wildlife Dept

Dr. Andy Schwartz, State Veterinarian and Executive Director, Texas Animal Health Commission

Shawn Schafer, Executive Director, North American Deer Farmers Association

Travis Lowe, Executive Director, North American Elk Breeders Association

Dr. Kelly Straka, DVM, MPH, Wildlife Section Manager, Minnesota Department of Natural Resources

Jason Sumner, Deputy Director, Missouri Department of Conservation

# Wednesday, May 31<sup>st</sup>, 2023

***Registration 7:30 AM - 6:00 PM***

## Welcome

8:00 - 8:20am Wednesday, 31st May, 2023  
Plaza Ballroom



Reid DeWalt, Assistant Director, Aquatic, Terrestrial, and Natural Resources, Colorado Parks and Wildlife



Alan Rudolph, Ph.D. Vice President for Research, Colorado State University

# Plenary Talk: Dr. Mark Zabel- Overcoming Barriers to Control CWD

8:20 - 8:40am Wednesday, 31st May, 2023

Plaza Ballroom



Dr. Zabel established his prion research laboratory at Colorado State University (CSU) eighteen years ago and is currently tenured Professor and Associate Director of the Prion Research Center in the Department of Microbiology, Immunology and Pathology and Research Associate dean in the College of Veterinary Medicine and Biomedical Sciences at CSU. He earned his PhD in experimental pathology from the University of Utah. Dr. Zabel was awarded the prestigious Human Frontiers in Science Long Term Research Fellowship and received postdoctoral training in prion biology, biochemistry and pathology from the laboratory of Dr. Adriano Aguzzi at the Neuropathology Institute, University Hospital of Zürich. He also received training in immunology in the laboratory of Nobel Prize laureate Dr. Rolf Zinkernagel in the Immunology Department, also at the University Hospital of Zürich.

Dr. Zabel is Associate Director of the Prion Research Center (PRC) at Colorado State University. The PRC fully embraces the emerging paradigm that all diseases and disorders with a protein misfolding etiology are prion diseases. The unique infrastructure of the PRC has enabled us to expand investigations beyond the classical prion diseases like Creutzfeld-Jakob disease, Kuru and Fatal Familial Insomnia; to investigate nonclassical prion diseases like Parkinson's and Alzheimer's diseases.

The Zabel laboratory employs molecular biological, immunological and biochemical techniques to explore peripheral pathogenesis, therapeutics and vaccines for prion diseases. Studying basic mechanisms of prion infection and dissemination provides translational information leading to diagnostic, therapeutic and vaccine targets. Dr. Zabel's research program focuses on the interaction of prions with cells and receptors of the immune system and lymphoid tissues in the early entry, trafficking, and pathogenesis phases of prion infections. He utilizes novel mouse models of Chronic Wasting Disease that have led to numerous publications detailing translational knowledge about the role of the Complement system in prion replication, disease progression and transmission.

# CWD Surveillance Strategies

8:40 - 10:20am Wednesday, 31st May, 2023

Plaza Ballroom

Moderator Dr. Qingzhong Kong

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8:40 - 9:00am

## Hierarchical Modeling and Data from Wind Cave National Park Provides Insight on Chronic Wasting Disease Diagnostics in Elk

Nathan Galloway<sup>1</sup>, Jenny Powers<sup>1</sup>, Glen Sargeant<sup>2</sup>, Gregory Schroeder<sup>3</sup>, Kaneesha Hemmerling<sup>4</sup>, Michelle Mousel<sup>5</sup>, Terry Spraker<sup>6</sup>, Margaret Wild<sup>4</sup>

<sup>1</sup>National Park Service Biological Resources Division, Fort Collins, USA. <sup>2</sup>US Geological Survey, Jamestown, USA. <sup>3</sup>Wind Cave National Park, Hot Springs, USA. <sup>4</sup>Washington State University, Pullman, USA. <sup>5</sup>US Department of Agriculture, Pullman, USA.

<sup>6</sup>Colorado State University, Fort Collins, USA

### Abstract

Both management and research of chronic wasting disease (CWD) depend upon reliable disease diagnostics. Postmortem immunohistochemistry (IHC) has long been recognized as the 'gold standard'. Enzyme-linked immunosorbent assay (ELISA; Bio-Rad) is also a highly sensitive and specific validated diagnostic assay for CWD. We compared IHC in rectal mucosa associated lymphoid tissue (RMALT), and both IHC and ELISA in medial retropharyngeal lymph node (RPLN) and brainstem at the level of the obex in free-ranging elk (*Cervus canadensis*) to clarify sensitivity and specificity of each.

We employed hierarchical Bayesian mixture models to predict diagnostic test performance parameters while including known sources of individual disease risk heterogeneity. We fit the model to data from 286 adult elk lethally removed for management objectives at Wind Cave National Park, South Dakota between 2016 and 2020. Of the 286 elk tested, 50 tested positive for CWD in at least one tissue. After including individual covariates for Prnp genotype and spatial sub-herd to account for heterogeneous disease risk, we predicted sensitivity and specificity of RPLN and obex by both ELISA and IHC, as well as RMALT IHC both at the level of the individual follicle and the individual elk. Both IHC and ELISA perform well, with lower credible intervals for test sensitivity predicted to be above 65% in all cases. Mean predicted test sensitivity was slightly higher for RPLN tissue than obex. For both the RPLN and obex, test specificity for ELISA was extremely high (0.98; 0.97-0.99) providing strong confidence in the truth of positive test results. For RMALT tissue, our data showed strong individual variation in the probability of a single follicle testing positive in a CWD-positive elk. When we subset the data to include only RMALT samples with follicle counts above a minimum as suggested by others, we show test sensitivity increases as minimum follicle count increases.

For the best CWD diagnostic performance in elk, managers and hunters should prioritize RPLN tissue but recognize that testing both obex and RPLN increases their ability to diagnose CWD. IHC may continue to serve as the gold standard, but ELISA positives are also very trustworthy and unlikely to be false. RMALT can also provide useful insight into disease ecology and distribution through antemortem testing.

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9:00 - 9:20am

## The "Big Data" models of the Surveillance Optimization Project for Chronic Wasting Disease [SOP4CWD] warehouse

Brenda Hanley<sup>1</sup>, Rachel Abbott<sup>1</sup>, Nicholas Hollingshead<sup>1</sup>, Cara Them<sup>2</sup>, Md Sohel Ahmed<sup>1</sup>, Krysten Schuler

<sup>1</sup>Cornell University, Ithaca, USA. <sup>2</sup>Cara Them Consulting, Corvallis, USA

## Abstract

Already discovered in cervids in 30 US states, with the potential to spread into every wild habitat that supports cervids and reduce hunter involvement, chronic wasting disease (CWD) poses a threat to the current operational framework of state wildlife agencies. We assume that management of such a wide-ranging and high-consequence disease requires a paradigm shift toward interagency partnerships and regional-scale, big data analyses. Housed in the Wildlife Health Lab of Cornell University, the Surveillance Optimization Project for Chronic Wasting Disease [SOP4CWD] has partnered with two dozen US state agencies to pool over ¾ of a million data points involving deer demography, disease testing, and landscape variables. We have archived the data into an online tool complete with preprogrammed models designed to answer a variety of scientific questions for agencies regarding what, where, and when of CWD infection in wild cervids. We discuss each of the SOP4CWD models and how they are capable of leveraging local and region data to pinpoint (1) locations with high risk of novel introduction, (2) areas with high potential for rapid spread resulting in an endemic disease, (3) anthropogenic, habitat, regulatory, or demographic, cofactors correlated disease events, and (4) strategies for surveillance and monitoring. Prior partnerships in SOP4CWD have been limited to those states and provinces east of the Mississippi, but additional agencies west of the Mississippi are now welcome to participate so we may all unlock the potential to learn from the single most comprehensive source of CWD data in North America.

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9:20 - 9:40am

## Can We Leverage Environmental Contamination for CWD Surveillance?

Miranda Huang<sup>1</sup>, Steve Demarais<sup>1</sup>, Alejandro Banda<sup>2</sup>, Bronson Strickland<sup>1</sup>, Anna Grace Welch<sup>2</sup>, Scoty Hearst<sup>3</sup>, Stuart Lichtenberg<sup>4,5</sup>, Allan Houson<sup>6</sup>, Kim Pepin<sup>7</sup>, Kurt VerCauteren<sup>7</sup>

<sup>1</sup>Mississippi State University, Starkville, USA. <sup>2</sup>Mississippi State University, Pearl, USA. <sup>3</sup>Mississippi College, Clinton, USA. <sup>4</sup>University of Minnesota, St. Paul, USA. <sup>5</sup>University of Wisconsin-Madison, Madison, USA. <sup>6</sup>University of Tennessee, Knoxville, USA. <sup>7</sup>National Wildlife Research Center, Fort Collins, USA

## Abstract

Chronic wasting disease (CWD) has impacted deer populations and upended wildlife management as it spreads across North America. One potential contributor to CWD environmental contamination is scraping behavior by white-tailed deer (*Odocoileus virginianus*, hereafter: WTD). Scraping communicates sociosexual status through disseminated bodily fluids (urine, saliva, glandular secretions) with the potential to spread infectious materials. We used data from 105 camera traps at scrapes over 4 months to document WTD visitation rates in southwestern Tennessee, an area with high CWD prevalence (~50%). We enhanced this analysis by testing prion levels in the soil of, and on the licking branch above, each scrape. Across 48 km<sup>2</sup>, we captured 3,063 scrape interactions by does, fawns, and 218 unique bucks. Scrapes were visited by an average of 12 unique bucks (standard deviation = 8, range = 1-39). We detected prion seeding activity in the soil of 20% and on the licking branches of 41% of Tennessee scrapes. We also collected soil and branch samples from scrapes in northern Mississippi, where CWD prevalence is lower (~8%). We detected prion seeding activity in the soil of 25% and on the licking branches of 11% of Mississippi scrapes. This environmental contamination at sites of natural deer congregation demonstrates the potential for prion exposure. Further, these results suggest scrapes could serve as environmental sentinels to identify CWD presence in an area without having to rely on testing harvested WTD. The potential for employing scrapes in the surveillance and management of CWD will be discussed.

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9:40 - 10:00am

## Identification of optimal training aids for chronic wasting disease detection dogs

Amritha Mallikarjun<sup>1</sup>, Ila Charendoff<sup>1</sup>, Madison Moore<sup>1</sup>, Michelle Gibson<sup>2</sup>, Elizabeth Nguyen<sup>1</sup>, Ben Swartz<sup>1</sup>, Julie Ellis<sup>2</sup>, Lisa Murphy<sup>2</sup>, Cynthia Otto<sup>1,3</sup>

<sup>1</sup>Penn Vet Working Dog Center, University of Pennsylvania, Philadelphia, USA. <sup>2</sup>Wildlife Futures Program, New Bolton Center, University of Pennsylvania, Philadelphia, USA. <sup>3</sup>Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania, Philadelphia, USA

## Abstract

Dogs can be trained to differentiate between CWD-positive and CWD-negative fecal samples and can find these samples in both laboratory and field settings (Mallikarjun et al., 2023). As such, operational detection dogs could be trained and deployed to identify areas containing CWD-positive fecal matter. This set of studies aimed to identify an alternate training aid for fecal samples that would not cause environmental contamination and would safely allow dogs to learn to CWD-positive fecal odor in locations like their target search areas.

In the first experiment, we tested a set of CWD detection dogs on their differentiation of CWD-positive and CWD-negative odor as contained in different potential training aids. The dogs were shown sets (CWD-positive, CWD-negative, and a control item) of potential alternate training aids (cotton balls, GetXent tubes, or PDMS) incubated at two different temperatures (21 C or 37 C) for three different periods of time (6 hrs, 24 hrs, or 48 hrs). Dogs showed the highest level of detection accuracy with cotton aids, and showed less false alerts with samples incubated at 21 C as compared to 37 C.

In the second experiment, a new set of dogs were trained on cotton aids incubated at 21 C for 24 hours. These dogs successfully learned the CWD odor on cotton, and more importantly identified the CWD odor in fecal matter despite never encountering CWD-positive fecal matter prior to test. Together, these experiments suggest that cotton aids can be used to safely train CWD detection dogs.

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10:00 - 10:20am

## 16 Detection of CWD prion in feces of naturally infected, pre-clinical, farmed North America white-tailed deer

Francisca Bravo-Risi<sup>1,2</sup>, Paulina Soto<sup>1,2</sup>, Rebeca Benavente<sup>1</sup>, Tracy A. Nichols<sup>3</sup>, Rodrigo Morales<sup>1,4</sup>

<sup>1</sup>Department of Neurology, The University of Texas Health Science Center at Houston, Houston, USA. <sup>2</sup>Universidad Bernardo O'Higgins, Doctorado en Ciencias con Mención en Materiales Funcionales, Santiago, Chile. <sup>3</sup>Veterinary Services Cervid Health Program, United States Department of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, USA. <sup>4</sup>Centro Integrativo de Biología y Química Aplicada (CIBQA), Universidad Bernardo O'Higgins, Santiago, Chile

## Abstract

Chronic wasting disease (CWD) is a prion disease affecting cervids. Confirmatory testing of CWD is performed postmortem in obex and head lymphoid tissues. Our group has explored CWD-prion detection in various sample types using the protein misfolding cyclic amplification (PMCA) technique including antemortem samples such as blood and semen. Previous studies demonstrate the presence of infectious prions in feces of CWD-infected deer using in vitro prion-amplification techniques and bioassays. In experimental conditions, this has been achieved as soon as 6-month post-inoculation in cervids, suggesting feces may be a candidate for antemortem testing. We optimized the detection of CWD-prions in fecal samples from naturally infected, pre-clinical white-tailed deer (WTD) by comparing enrichment of CWD-prions by NaPTA, ultracentrifugation, and direct spiking of the sample to the PMCA reactions demonstrating that CWD-prion detection in feces is best in the absence of sample pre-treatments. The PMCA screening results of 169 fecal samples were compared with those previously published on blood. The detection of CWD-prions by PMCA in a late pre-clinical stage was similar for both sample types: 84% for feces and 87% for blood. These results contrasted with a lower detection observed at early pre-clinical stages (28% and 47% for feces and blood, respectively). Importantly, our analysis also considered the genetic variability at position 96 of the prion protein and sex. Overall, our findings contribute to understand prion distribution across different biological samples and polymorphic variants of WTD. This information is relevant for the current effort to identify platforms to diagnose CWD.

## BREAK

10:20 - 10:40am Wednesday, 31st May, 2023  
Plaza Exhibit Foyer

## CWD Surveillance Strategies Continued

10:40am - 12:00pm Wednesday, 31st May, 2023  
Plaza Ballroom

Moderator Dr. Qingzhong Kong

10:40 - 11:00am

## **Canine biodetection of an odor signature in white-tailed deer associated with infection by Chronic Wasting Disease prions.**

Glen Golden<sup>1</sup>, Elizabeth Ramirez<sup>1</sup>, Hayley Stevens<sup>1</sup>, Amelia Johnston<sup>1</sup>, Tracy Nichols<sup>2</sup>, Daniel Grove<sup>3,4</sup>, Richard Bowen<sup>1</sup>, Thomas DeLiberto<sup>5</sup>, Jennifer Bourbois<sup>1</sup>, Bruce Kimball<sup>6</sup>

<sup>1</sup>Colorado State University, Fort Collins, USA. <sup>2</sup>Cervid Health MRP APHIS VS, Fort Collins, USA. <sup>3</sup>University of Tennessee, Knoxville, USA. <sup>4</sup>Tennessee Wildlife Resources Agency, Knoxville, USA. <sup>5</sup>MRP APHIS, Fort Collins, USA. <sup>6</sup>Monell Chemical Senses Center, Philadelphia, USA

### **Abstract**

Chronic wasting disease (CWD) has become a major concern amongst those involved in managing wild and farmed cervid populations. CWD management is complicated by the lack of a practical, non-invasive, live-animal screening tests. Studies have shown animal biodetectors can detect changes in odor based on infection status despite environmental variation. In previous experiments, we have demonstrated that mice, ferrets, and most recently, dogs can detect avian influenza (AI) infection using fecal, gastrointestinal tract, and cloacal swab volatile odor compounds. We found that dogs (n=6) can detect the metabolic result of being infected with the CWD prion, in fecal samples collected from white-tailed deer. Importantly, these dogs have the capability to detect small intestine tissue collected from confirmed CWD infected deer showing the potential to detect CWD infection based on whole body odor profiles. Our current study clearly showed that canine biodetectors can be trained to identify populations and/or individuals infected with CWD prions via detection of fecal, colon, and small intestine odors in the laboratory and potentially in the field. Such a tool may also prove useful in identifying infected live animals, carcasses, urine, feces, and contaminated environments.

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11:00 - 11:20am

## **A Microfluidic MEMS Biosensor for Rapid and Accurate Detection of Chronic Wasting Disease Pathologic Protein**

Sura A. Muhsin<sup>1</sup>, Amjed Abdullah<sup>1</sup>, Estela Kobashigawa<sup>2</sup>, Sherri Russell<sup>3</sup>, Michael Zhang<sup>2</sup>, Shuping Zhang<sup>4</sup>, Jasmine Batten<sup>5</sup>, Mahmoud Almasri<sup>6</sup>

<sup>1</sup>Department of Electrical and Computer Engineering University of Missouri, Columbia, USA. <sup>2</sup>College of Veterinary Medicine, Columbia, USA. <sup>3</sup>Missouri Department of Conservation, Missouri, USA. <sup>4</sup>College of Veterinary Medicine, University of Missouri, Columbia, USA. <sup>5</sup>Wisconsin Department of Natural Resources, Madison Wisconsin, USA. <sup>6</sup>Department of Electrical and Computer Engineering, Columbia, USA

### **Abstract**

Chronic Wasting Disease (CWD) disease development involves a conformational conversion of the cellular prion protein to a protease-resistant and infectious conformer. The disease has proven to be difficult to control in part because of the sensitivity and specificity issues with the current test regime. We have developed a microfluidic-based impedance biosensor for rapid detection of CWD pathologic prion protein using positive platelet prion samples, positive animal lymph node tissue samples, and positive antigen control samples that are included in the CWD ELISA IDEXX kit. The device is unique in terms of its ability to concentrate low quantities of CWD prions to a detectable level, trap and detect the prions with high sensitivity, and selectivity in 40 minutes. The sensing electrode was precoated with a mixture of anti-prion mAB (F89/160.1.5) and cross-linker (Sulfo-LC-SPDP). We have demonstrated focusing and trapping capabilities using beads with (diameter <0.5 µm) and 4 Vp-p, 5 MHz for focusing, and 5 Vp-p at 6 MHz for trapping. The positive platelet prion samples were used as is, the CWD positive control antigen was subjected to 2-fold dilution (1:4 – 1:24) while the RPLN homogenates (30 ELISA-positive and 30-ELISA negative) were subjected to 10-fold serial dilutions (10<sup>-1</sup> to 10<sup>-4</sup>). The specificity testing for pathogenic prion was confirmed by proteinase K treatment of RPLN homogenates prior to loading the samples into the biosensor. We compare to existing platforms and discuss next steps in evaluation of this potentially practical option.

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11:20 - 11:40am

## Prion Forensics: a multidisciplinary approach to investigate chronic wasting disease at a deer carcass disposal site

Marc D. Schwabenlander<sup>1,2</sup>, Jason C. Bartz<sup>3</sup>, Michelle Carstensen<sup>4</sup>, Alberto Fameli<sup>5</sup>, Linda Glaser<sup>6</sup>, Roxanne J. Larsen<sup>1,2</sup>, Mancu Li<sup>1,2</sup>, Laramie L. Lindsey<sup>1,2</sup>, Jonathan D. Oliver<sup>7</sup>, Rachel L. Shoemaker<sup>1,2</sup>, Gage Rowden<sup>1,2</sup>, Suzanne Stone<sup>1,2</sup>, W. David Walter<sup>8</sup>, Tiffany M. Wolf<sup>9,2</sup>, Peter A. Larsen<sup>1,2</sup>

<sup>1</sup>Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, USA.

<sup>2</sup>Minnesota Center for Prion Research and Outreach, College of Veterinary Medicine, University of Minnesota, St. Paul, USA.

<sup>3</sup>Department of Medical Microbiology and Immunology, School of Medicine, Creighton University, Omaha, USA. <sup>4</sup>Minnesota Department of Natural Resources; Wildlife Health Program, Forest Lake, USA. <sup>5</sup>Pennsylvania Cooperative Fish & Wildlife Research Unit, The Pennsylvania State University, University Park, USA. <sup>6</sup>Minnesota Board of Animal Health, St. Paul, USA. <sup>7</sup>Division of Environmental Health Sciences, School of Public Health, University of Minnesota, Minneapolis, USA. <sup>8</sup>U.S. Geological Survey, Pennsylvania Cooperative Fish and Wildlife Research Unit, The Pennsylvania State University, University Park, USA. <sup>9</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, USA

### Abstract

Chronic wasting disease (CWD) is confirmed in 30 US states, three Canadian provinces, Nordic countries, and South Korea. Although the origin and progression are typically unknown, CWD spread over the past seven decades is attributed, in part, to cervid carcass transport and disposal. Given the potential for CWD-causing prions to resist degradation and remain infectious within the environment, the disposal of CWD-positive animal remains, whether from free-ranging or captive sources, can play an important role in the transmission of CWD. Management agencies provide disposal guidance and opportunities to reduce the risk of introduction to new areas. Upon the discovery of an illegal carcass disposal site associated with a CWD-positive captive cervid facility, we leveraged an integrative multidisciplinary approach of anatomic, entomologic, genetic, and prion amplification methods to discover multiple CWD-positive remains of white-tailed deer across several age classes and, using microsatellite markers, we confirmed a portion of these remains originated from the CWD infected captive herd. CWD prions were detected via RT-QuIC in 14 of 56 carcass samples, including fly larvae associated with the remains. Our multi-methods approach, coined as "Prion Forensics", provides the foundation for future investigations of prion transmission risk from carcass disposal.

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11:40am - 12:00pm

## Progress on a Live Animal RT-QuIC Test for Chronic Wasting Disease

Davin Henderson<sup>1</sup>, Deepanker Tewari<sup>2</sup>, Melinda Fasnacht<sup>2</sup>, Margaret Ritzman<sup>2</sup>, Julia Livengood<sup>2</sup>, Jessica Bower<sup>2</sup>, Alex Hamburg<sup>2</sup>, Kevin Brightbill<sup>2</sup>

<sup>1</sup>CWD Evolution LLC, Fort Collins, USA. <sup>2</sup>Pennsylvania Veterinary Laboratory, Harrisburg, USA

### Abstract

Chronic wasting disease (CWD) is an expanding prion disease affecting cervids in North America, Northern Europe and the Korean Peninsula. Diagnosis of CWD currently relies on immunohistochemical analysis of the brain stem/obex or the retropharyngeal lymph node (RPLN). Immunohistochemistry relies on the relative stability of CWD prions to differentiate disease associated PrPCWD from the cellular, and naturally occurring PrPC. More recently, amplification methods which harness the replicative nature of prions have been applied to the detection of CWD with log-fold improvements in sensitivity. Real-time quaking induced conversion (RT-QuIC), has emerged as a semiquantitative, sensitive and specific test for the detection of CWD in both tissues harvested post-mortem and ante-mortem. Numerous studies have reported the utility of RT-QuIC for live animal testing. Here, we report recent sensitivity and specificity of RT-QuIC rectal biopsy testing on naturally infected white-tailed deer compared to gold standard post-mortem diagnosis of CWD. We saw a sensitivity of 85.7% (n = 71) in rectal biopsy RT-QuIC testing which increased to 94.7% in deer that were IHC positive for CWD in both RPLN and obex. We also compared the sensitivity of fecal RT-QuIC testing compared to post-mortem IHC analysis and saw a sensitivity of 60% (n = 69) which increased to 70.5% when both postmortem tissues were positive by IHC. Specificity for both testing methodologies was 100%. The use of environmental fecal RT-QuIC CWD surveillance in both captive cervid operations and in the wild may help predict the presence of CWD without culling or capture.



# LUNCH

12:00 - 1:20pm Wednesday, 31st May, 2023

Local restaurants can be found at

*[www.visitDenver.com](http://www.visitDenver.com)*

## CWD Management

1:20 - 2:40pm Wednesday, 31st May, 2023  
Plaza Ballroom

Moderator Jason Sumner

1:20 - 1:40pm

### **Elk are not just big deer: consideration of host differences in chronic wasting disease**

Margaret Wild<sup>1</sup>, Glen Sargeant<sup>2</sup>, Jenny Powers<sup>3</sup>, Nathan Galloway<sup>3</sup>, Terry Spraker<sup>4</sup>

<sup>1</sup>Washington State University, Pullman, USA. <sup>2</sup>U.S. Geological Survey, Northern Prairie Wildlife Research Center, Jamestown, USA.

<sup>3</sup>National Park Service, Biological Resources Division, Fort Collins, USA. <sup>4</sup>Colorado State University, Fort Collins, USA

#### **Abstract**

Chronic wasting disease (CWD) is a transmissible prion disease of cervids. Although knowledge of CWD has increased markedly during the past two decades, much remains poorly understood regarding host, prion, and environmental factors that facilitate transmission. Most studies on CWD in cervids have been conducted with white-tailed (*Odocoileus virginianus*) and mule deer (*O. hemionus*). Findings in one cervid species are often extrapolated to others to efficiently fill knowledge gaps; however, consideration should also be given to differences among species, which should temper generalizations. For example, data do not support a generalization that CWD prevalence is always lower in elk (*Cervus canadensis*) than in sympatric deer. Similarly, although CWD prevalence in male deer is higher than females, a sex difference has not been documented in elk. Differences are also evident in the amount and distribution of prion accumulation in tissues, particularly lymphoid tissues, of elk and deer, which suggest potential differences in host prion processing or among prion strains. Such differences also influence the reliability of CWD diagnoses, particularly tests of tissues that can be obtained antemortem. Variations in life histories, social behavior, and land use of species should also be considered in regard to species differences in CWD transmission and epidemiology. For example, whereas white-tailed deer show strong fidelity to matrilineal groups, elk congregate apparently interchangeably in groups of variable and changing size and distribution. Finally, where CWD prevalence in elk is high, effects on population growth may be exacerbated by an intrinsically lower recruitment rate in elk; consequently, CWD prevalence of 13% was associated with zero population growth even in the absence of harvest. Effects of CWD on elk may thus be underappreciated. Continued investigation is needed to understand general, as well as species specific, features and impacts of CWD on cervids.

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1:40 - 2:00pm

### **Spatially variable effects of chronic wasting disease on elk populations: implications for monitoring and management**

Glen Sargeant<sup>1</sup>, Margaret Wild<sup>2</sup>, Nathan Galloway<sup>3</sup>, Jenny Powers<sup>4</sup>, Angela Jarding<sup>5</sup>, Greg Schroeder<sup>5</sup>

<sup>1</sup>U.S. Geological Survey, Northern Prairie Wildlife Research Center, Jamestown, USA. <sup>2</sup>College of Veterinary Medicine, Washington State University, Pullman, USA. <sup>3</sup>National Park Service, Biological Resources Division, Fort Collins, USA. <sup>4</sup>National Park Service, Biological Resources Division, Fort Collins, USA. <sup>5</sup>National Park Service, Wind Cave National Park, Hot Springs, USA

## Abstract

In the western United States, elk and deer range typically is partitioned into strata known colloquially as game management units (GMUs). Such strata are used as geographic bases for harvest management and, importantly, for monitoring and management of chronic wasting disease (CWD). Although GMUs often are relatively homogeneous with respect to stakeholder interests, landscape features, and vital rates, prevalence of chronic wasting disease may vary by an order of magnitude at a much finer scale of geographic resolution. We used locations obtained with Global Positioning System (GPS) collars and CWD tests of culled elk to partition Wind Cave National Park (WICA) into 3 22–56 km<sup>2</sup> spatial strata used by distinct subsets of an unhunted elk population. Although strata were adjacent and not much larger than ranges of individual elk, CWD prevalence varied among strata from 0.03 to 0.29. We used GPS locations of marked elk and stratum-specific survival records to relate CWD prevalence to mortality, project changes in relative abundance of population subsets, and describe effects of changing relative abundance on fidelity of elk to group ranges. When CWD prevalence is spatially variable, aggregate statistics based on opportunistic collection of tissue samples from hunter-killed elk and deer reflect the geographic distribution of harvest. Consequently, changes in cervid distribution or hunter access may affect aggregate estimates of CWD prevalence even when local prevalence of CWD has not changed. Our results highlight needs for precise reporting of harvest locations and for consideration of spatial heterogeneity when hypotheses are formulated and data are analyzed.

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2:00 - 2:20pm

## What the parts can tell us about the whole: using a holistic approach to disentangle the complexity of CWD management in Wisconsin

Daniel Walsh<sup>1</sup>, Tom Fiddaman<sup>2</sup>, LeAnn White<sup>3</sup>, Scott Hull<sup>4</sup>, Tami Ryan<sup>4</sup>, David MacFarland<sup>4</sup>, Daniel Storm<sup>4</sup>, Jasmine Batten<sup>4</sup>, Bryan Richards<sup>3</sup>

<sup>1</sup>USGS Montana Cooperative Wildlife Research Unit, Missoula, MT, USA. <sup>2</sup>Ventana, Inc., Bozeman, MT, USA. <sup>3</sup>USGS National Wildlife Health Center, Madison, WI, USA. <sup>4</sup>Wisconsin Department of Natural Resources, Madison, WI, USA

## Abstract

Beyond the complex ecological and epidemiological interactions influencing Chronic Wasting Disease (CWD) in free-ranging populations, efforts to monitor and manage this disease have been complicated by social, economic and political factors that affect intervention opportunities, consequences for stakeholders, and ultimately disease outcomes. Deer and disease dynamics can also influence ecosystem structure with potential long-term negative influences on biodiversity and ecological stability. Navigating this complex system is challenging for natural resource agencies as unintended and unanticipated consequences of well-intentioned management actions can undermine stakeholder support. To overcome these challenges, the U.S. Geological Survey (USGS) National Wildlife Health Center and Montana Cooperative Wildlife Research Unit, Ventana Systems, Inc., and the Wisconsin Department of Natural Resources (WDNR) are applying a systems approach to map and model the complex relationships among ecological, epidemiological, social, and political processes affecting CWD. Through participatory modeling, we have gathered expert input on epidemiology, social science, and deer and forest health, integrating this wealth of existing knowledge with empirical data collected by the WDNR on the ecological and social processes influencing CWD dynamics and management. Initial findings from the model, parameterized with this information, include (1) status quo management is characterized by approximately 25% annual growth, (2) arresting growth requires large reductions in transmission, on the order of 50-80%, (3) no single management policy can achieve stabilization or disease reduction in Wisconsin, but a suite of intensive and prolonged actions may, (4) some necessary actions are unavailable due to jurisdiction and resource constraints, and (5) in low prevalence and new introduction areas, timely application of interventions is important as disease growth and response metrics are not linear. To make this model more accessible, we developed a user-friendly management flight simulator that allows natural resource managers and stakeholders to visualize, in real time, both short- and long-term impacts of management decisions on deer population dynamics, disease processes, stakeholders' response to management, and the down-stream impacts on the forest system. The model is extensible; USGS and Ventana's future plans include incorporating management frameworks across multiple jurisdictions and affected cervid species.

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2:20 - 2:40pm

## **Bioeconomic Tool: harvest strategies for Chronic Wasting Disease management in Alberta**

Evelyn Merrill<sup>1</sup>, Anne Hubbs<sup>2</sup>, Vic Adamowicz<sup>3</sup>, Marty Luckert<sup>3</sup>, Qin Xu<sup>3</sup>, Maria Dobbin<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, University of Alberta, Edmonton, Canada. <sup>2</sup>Alberta Fish and Wildlife Stewardship, Government of Alberta, Rocky Mountain House, Canada. <sup>3</sup>Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, Canada

### **Abstract**

Chronic wasting disease (CWD) produces population-level declines in wild cervids, with associated ecological and socio-economic repercussions. To date, harvest management is the primary approach to reduce CWD transmission and spread. We present an Integrated Population Modeling approach (IPM) for developing the scientific basis for adaptive, harvest policies for managing CWD in mule deer in Alberta. Inputs to the model include mule deer population numbers from aerial surveys, mule deer demographic, harvest data, and hunter preferences from hunter-harvest surveys, mule deer population processes from field studies, and disease prevalence data from the Alberta CWD surveillance program between 2000 – 2022. We provide an overview of the structure of and components of the mule deer IPM and issues related to how CWD transmission is incorporated into the IPM. We give an overview of how the IPM is integrated into a Bioeconomic Tool for assessing the cost-benefit of a suite of CWD harvest strategies for maintaining deer populations and hunting opportunities while minimizing CWD prevalence and spread. Finally, we illustrate how strong research collaboration, including an Advisory Committee of Alberta experts, helps to ensure data rigor and standardization, and deer management application, including recommendations for future data collection and recording practices

## **BREAK**

2:40 - 3:00pm Wednesday, 31st May, 2023  
Plaza Exhibit Foyer

## **CWD in the Environment**

3:00 - 4:40pm Wednesday, 31st May, 2023  
Plaza Ballroom

Moderator Dr. Dan Walsh

3:00 - 3:20pm

## **Chronic wasting disease (CWD) detection in environmental and biological samples from a taxidermy site**

Paulina Soto<sup>1,2</sup>, J Hunter Reed<sup>3</sup>, Mitch Lockwood<sup>4</sup>, Rodrigo Morales<sup>1,2</sup>

<sup>1</sup>Department of Neurology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, USA.

<sup>2</sup>Universidad Bernardo O'Higgins, Santiago, Chile. <sup>3</sup>Texas Parks and Wildlife Department, San Antonio, USA. <sup>4</sup>Texas Parks and Wildlife Department, Kerrville, USA

## Abstract

Chronic wasting disease (CWD) is a transmissible prionopathy affecting free-ranging and captive deer which can spread through both direct and indirect transmission. One area of concern is the risk of CWD transmission associated with taxidermy operations, especially since most CWD susceptible species brought to these operations are of unknown CWD status. Furthermore, taxidermy facilities can become a nidus of prion infectivity if biosecurity practices are not followed or implemented. In this study, we evaluated the presence of infectious prions in a taxidermy facility that was possibly exposed to CWD. To determine if the facility was exposed to CWD, we collected biological and environmental specimens from the facility, and we screened the samples for CWD prions through the protein misfolding cyclic amplification (PMCA) technique. Additionally, we swabbed different surfaces possibly exposed to CWD-infected animals or carcasses. For the PMCA reactions, we directly used a swab piece or 10  $\mu$ L of 20% w/v homogenized samples. We detected the presence of prions in i) soils that were in contact with the heads of dead animals, ii) insects used to clean skulls, and iii) dumpsters where animal carcasses were disposed. This is the first report demonstrating that environmental swabbing is a useful surveillance method to screen for CWD-prion infectivity. In addition, our results suggest that CWD may be transmitted due to taxidermy practices.

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3:20 - 3:40pm

## Real-time quaking-induced conversion for prion detection in contaminated environmental samples

Stuart Lichtenberg<sup>1,2,3</sup>, Samuel Thomas<sup>3</sup>, Daniel Storm<sup>4</sup>, Daniel Walsh<sup>5</sup>

<sup>1</sup>University of Minnesota Department of Veterinary and Biomedical Sciences, St. Paul, USA. <sup>2</sup>University of Minnesota Prion Research and Outreach Center, St. Paul, USA. <sup>3</sup>University of Wisconsin-Madison Department of Soil Science, Madison, USA. <sup>4</sup>Wisconsin Department of Natural Resources, Eau Claire, USA. <sup>5</sup>U.S. Geological Survey, Montana Cooperative Wildlife Research Unit, University of Montana, Missoula, USA

## Abstract

Chronic wasting disease (CWD) is unique among prion diseases in that it affects free-ranging host species. Consequently, indirect CWD transmission via prions shed by diseased animals into the broader environment is of great concern. Prions in the environment may contribute to further geographic spread of the disease, but adequate surveillance—a first step in management—is hampered by the lack of fit-for-purpose analytical methods. Antibody-based methods have limits of detection far above infection-relevant concentrations likely to be disbursed into the environment, and bioassays have experimental duration on the order of years. We describe here a method for extraction of prions from environmental matrices which are then amenable to detection by the real-time quaking induced conversion (RT-QuIC) assay. Furthermore, we characterize assay performance attributes (e.g., influence of organic matter) and demonstrate detection of prions from soil samples with varying properties.

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3:40 - 4:00pm

## Sensitive detection of swab-recovered chronic wasting disease prions from environmentally relevant surfaces

Qi Yuan<sup>1</sup>, Gage Rowden<sup>2</sup>, Tiffany Wolf<sup>2</sup>, Marc Schwabenlander<sup>2</sup>, Peter Larsen<sup>2</sup>, Shannon Bartelt-Hunt<sup>3</sup>, Jason Bartz<sup>1</sup>

<sup>1</sup>Creighton University, Omaha, USA. <sup>2</sup>University of Minnesota, Saint Paul, USA. <sup>3</sup>University of Nebraska-Lincoln, Omaha, USA

## Abstract

Chronic wasting disease (CWD) has been identified in 30 states in the United States, four provinces in Canada, and recently in Scandinavia. Environmental elements such as soil, plants, and surfaces can harbor CWD prions for years and become persistent prion sources which exacerbate disease transmission. Therefore, it is critical to identify CWD prions in the environment to help monitor prion contamination and control disease transmission. An efficient method for CWD prion detection from contaminated environmental surfaces does not exist. In this study, we developed a rapid method for extracting prions from swabs using mechanical power and

quantified prion recovery from different types of surfaces including glass, stainless steel, and wood. We found that drying of prions on swabs were unfavorable for extraction, requiring stronger mechanical power to achieve higher recovery. We found the recovery of surface-dried CWD prions were approximately 30% from glass and stainless steel, whereas wood-recovered CWD prions was below the detection limit of 96-well immunoassay. To detect surface-recovered prions beyond the detection limit of the immunoassay, we used real-time quaking-induced conversion (RT-QuIC), an ultrasensitive method, and found an increase of 4 orders of magnitude for CWD prions recovered from stainless steel. More importantly, the RT-QuIC seeding activity of stainless steel-recovered CWD prion was similar ( $< 1$  Log) to that directly added to the RT-QuIC reaction. Our findings provided a rapid, ultrasensitive method for prion detection from contaminated surfaces which can be applied to monitor prion contamination in both environmental and medical settings.

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4:00 - 4:20pm

## **Application of Methods for Detecting Environmental Prion Protein (ePrP) Via Surfaces to Managing Chronic Wasting Disease**

Marc D Schwabenlander<sup>1,2</sup>, Catalina Picasso-Risso<sup>1,2</sup>, Sarah C. Gresch<sup>1,2</sup>, Marissa S. Milstein<sup>1,2</sup>, Gage Rowden<sup>1,2</sup>, Erik Hildebrand<sup>3</sup>, Patrick Hagen<sup>4</sup>, Mitch Lockwood<sup>5</sup>, Joseph Hediger<sup>6</sup>, Michael J. Cherry<sup>6</sup>, David G. Hewitt<sup>6</sup>, Qi Yuan<sup>7,2</sup>, Jason C. Bartz<sup>7,2</sup>, Tiffany M. Wolf<sup>8,2</sup>, Peter A. Larsen<sup>1,2</sup>

<sup>1</sup>Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA. <sup>2</sup>Minnesota Center for Prion Research and Outreach, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA. <sup>3</sup>Minnesota Department of Natural Resources, Forest Lake, MN, USA. <sup>4</sup>Minnesota Department of Natural Resources.mn.us, Forest Lake, MN, USA. <sup>5</sup>Texas Parks and Wildlife Department, Kerrville, TX, USA. <sup>6</sup>Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville, TX, USA. <sup>7</sup>Department of Medical Microbiology and Immunology, School of Medicine, Creighton University, Omaha, NE, USA. <sup>8</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

### **Abstract**

With increasing incidence and geographic spread of chronic wasting disease (CWD) across North America and Europe, developing highly sensitive misfolded prion (PrP<sup>Sc</sup>) detection methodologies in order to combat CWD is urgently needed. Real-time quaking-induced conversion (RT-QuIC) is one such assay for PrP<sup>Sc</sup> detection. Similar to environmental DNA (eDNA) advances for pathogen detection and species discovery in aquatic and terrestrial environments, our work investigates a rapid method for extracting and detecting prions from environmental surface swabs when paired with RT-QuIC. We applied our laboratory-based methods in three scenarios where environmental PrP<sup>Sc</sup> detection can enhance early CWD detection - captive cervids, wild cervid targeted surveillance, and venison processing. We deployed surfaces known to bind PrP<sup>Sc</sup> as environmental prion protein (ePrP) sentinels in food sources of herds with variable CWD prevalence. We detected PrP<sup>Sc</sup> in natural settings using swabbing and extraction methods in conjunction with RT-QuIC. CWD prevalence of a captive deer herd correlated with intensity of PrP<sup>Sc</sup> detection. For instance, 1 of 16 swabs were RT-QuIC positive in a pen with 1 of 12 immunohistochemistry (IHC) positive animals. Further, in another pen with 13 of 19 IHC positive animals, 19 of 34 swabs were RT-QuIC positive. Importantly, we identified potential environmental factors (e.g., feed type) that affected RT-QuIC results. Initial results of venison processing surfaces indicated PrP<sup>Sc</sup> can be detected after processing CWD-positive deer muscle. Furthermore, 10% bleach treatment eliminates detection. Our findings open the possibility for ePrP detection through non-invasive methods for early detection in CWD surveillance and management.

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4:20 - 4:40pm

## **Chronic wasting disease prions in soils: fate and detection**

Alsu Kuznetsova<sup>1</sup>, Erin Moffat<sup>2</sup>, Trent Bollinger<sup>2</sup>, Bjørnar Ytrehus<sup>3</sup>, Kjersti Selstad Utaaker<sup>3</sup>, Charlie Bahnson<sup>4</sup>, Debbie McKenzie<sup>5</sup>, Judd M. Aiken<sup>5</sup>

<sup>1</sup>University of Alberta, EDMONTON, Canada. <sup>2</sup>University of Saskatchewan, Saskatoon, Canada. <sup>3</sup>Norwegian Institute for Nature Research (NINA), Trondheim, Norway. <sup>4</sup>North Dakota Game and Fish, Bismarck, USA. <sup>5</sup>University of Alberta, Edmonton, Canada

## Abstract

A remarkable property of CWD prions (PrPCWD) is their persistence in external environments and ability to remain infectious for years. Soils are a natural environmental reservoir of shed PrPCWD that increasing the risk for disease transmission, both within and between species. Environmental transmission of CWD via soil depends on soil properties: it is facilitated in the prairie regions, while lowered reduced bioavailability of environmental PrPCWD in boreal, tundra and alpine soils may reduce the efficiency of indirect transmission. Identification of CWD prions in soils would have numerous benefits, including monitoring spread and infectivity persistence. Prion detection in soils is challenging as recovery of soil-bound PrPCWD is influenced by soil texture, mineralogy, humus content and becomes more difficult with time.

We developed a reliable, sensitive method to detect PrPCWD in different type of soils with a level of detection, when soil bound, of  $10^{-5}$ - $10^{-7}$  µg/µl. Soil surface horizon samples were collected from CWD-endemic regions with low and high CWD prevalence including prairie soils from North Dakota and southern Saskatchewan as well as boreal/alpine soils from Norway. Prions were extracted from soils and used as a seed for serial PMCA. PrPCWD were most commonly detected in soils from regions of high CWD prevalence (Saskatchewan) and not detected in prairie soils sampled from regions of low prevalence (North Dakota). PrPCWD were also amplified from soil samples of low CWD prevalence but where animals were concentrated near salt licks (Norway). This represents a significant improvement in soil-bound PrPCWD detection benefiting both surveillance and mitigation approaches.

## POSTER SESSION & HAPPY HOUR

4:40 - 6:15pm Wednesday, 31st May, 2023  
Plaza Exhibit Foyer

Two drink tickets are included in registration packets

### Posters:

### 3 Are Hunters Concerned About the Prevalence Rate of Chronic Wasting Disease in Newly Affected States?

Catherine Cummings<sup>1</sup>, Stephen S. Ditchkoff<sup>1</sup>, William Gulsby<sup>1</sup>, Ryan Williamson<sup>2</sup>, Kelly H. Dunning<sup>1</sup>

<sup>1</sup>Auburn University College of Forestry, Wildlife, and Environment, Auburn, USA. <sup>2</sup>Auburn University Department of Political Science, Auburn, USA

### Abstract

Chronic Wasting Disease (CWD) is a 100% fatal neurological disease that affects members of the deer family, Cervidae. CWD has been found in 30 states across the United States, making it a management issue that has garnered national attention. The 2021-2022 deer hunting season resulted in four new states identifying CWD-positive animals. These new emergences of CWD could negatively affect hunting participation and license sales, and thereby funding for conservation and the economies of rural communities. In this study, we aim to determine if Alabama and Louisiana deer hunters were concerned about the prevalence rate of CWD in their respective state. An online questionnaire was distributed to deer hunters in Alabama (n =689) and Louisiana (n = 682) with the sample obtained from online hunting forums and email lists. Several logistic regressions were used to determine which factors might predict whether a hunter is concerned about CWD prevalence rates. Alabama deer hunters were statistically significantly more concerned about the prevalence rate of CWD if they were older and more educated with hunting motivations that included experiencing nature and managing deer populations. Louisiana deer hunters were statistically significantly more concerned about the prevalence rate of CWD if they harvested fewer deer in the 2021-2022 season and if their hunting motivations were to manage deer populations and to harvest deer for consumption. As CWD will likely continue to spread, results from this study can help state agencies respond to a positive detection with scientifically and socially sound management strategies.

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## 6 Evaluation of real-time quaking-induced conversion for prion strain discrimination and infectivity determination

Jay Hrdlicka, Qi Yuan, Jason Bartz Creighton University, Omaha, USA

### Abstract

Chronic wasting disease (CWD) is a prion disease that affects cervids, is inevitably fatal, highly contagious, with an unknown host range. Real-time quaking-induced conversion (RT-QuIC) has proven as a reliable assay in detecting amyloid fibril formation and diagnosing prion diseases, but there is limited information about the use of RT-QuIC to quantify prion infectivity and strain discrimination. While animal bioassay remains the gold standard in assessing these metrics, it is burdensome with respect to animals, time, and cost. Evidence supporting the emergence of distinct CWD prion strains, has heightened the need for a more practical method capable of quantifying said metrics. We hypothesize RT-QuIC can quantify prion infectivity and discriminate prion strains. To investigate the ability of RT-QuIC in assessing prion infectivity, we compared the intracerebral bioassay lethal dose (LD<sub>50</sub>) to the RT-QuIC seeding dose (SD<sub>50</sub>) value that is defined as the dose of prions resulting in a positive signal in RT-QuIC for 50% of tested samples. To investigate the ability of RT-QuIC to discriminate strains, we compared the SD<sub>50</sub> difference and LD<sub>50</sub> difference between the two hamster-adapted prion strains hyper transmissible mink encephalopathy (HY TME) and drowsy transmissible mink encephalopathy (DY TME). We found RT-QuIC accurately assessed infectivity for HY TME but not for DY TME. Further comparison of SD<sub>50</sub> values to LD<sub>50</sub> values, demonstrated the ability of RT-QuIC to discriminate between the two distinct hamster-adapted prion strains, exemplifying the potential for RT-QuIC as a valuable tool in discriminating emerging CWD prion strains.

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## 8 The chronic wasting disease agent from white-tailed deer is infectious to humanized mice after passage through raccoons

Eric Cassmann<sup>1</sup>, Xu Qi<sup>2</sup>, Qingzhong Kong<sup>2</sup>, Justin Greenlee<sup>1</sup>

<sup>1</sup>USDA ARS, Ames, USA. <sup>2</sup>Case Western Reserve University, Cleveland, USA

### Abstract

The aim of this study was to evaluate the zoonotic potential of the raccoon passaged chronic wasting disease (CWD) agent in humanized transgenic mice in comparison with the North American CWD agent from the original white-tailed deer (WTD) host. Pooled brain (GG96) from CWD positive white-tailed deer was used to intracranially inoculate two WTD and one raccoon. Brain homogenates (10% w/v) from the raccoon and the WTD were used to intracranially inoculate transgenic mice (Tg40h) expressing the methionine 129 human prion protein. Brains and spleens were collected from mice at experimental endpoints of clinical disease or approximately 700 days post-inoculation. Tissues were divided and homogenized or fixed in 10% buffered neutral formalin. Immunohistochemistry, enzyme immunoassay, and western blot were used to detect misfolded prion protein (PrP<sup>Sc</sup>) in tissue. Tg40h mice inoculated with the raccoon passaged CWD agent from WTD exhibited a 100% (12/12) attack rate with an average incubation period of 605 days. PrP<sup>Sc</sup> was detected in brain tissue by enzyme immunoassay with an average optical density of 3.6/4.0 for positive brains. PrP<sup>Sc</sup> also was detected in brain tissue by western blot and immunohistochemistry. No PrP<sup>Sc</sup> was detected in the spleens of mice inoculated with the raccoon passaged CWD agent. Humanized mice inoculated with the CWD agent from WTD did not have detectable PrP<sup>Sc</sup> using conventional immunoassay techniques. These results demonstrated that the host range of the CWD agent from WTD was expanded in our experimental model after one passage through raccoons.

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## 9 Carrot plants as potential vectors for CWD transmission.

Paulina Soto<sup>1,2</sup>, Francisca Bravo-Risi<sup>1,2</sup>, Claudio Soto<sup>1</sup>, Rodrigo Morales<sup>1,2</sup>

<sup>1</sup>Department of Neurology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, USA.

<sup>2</sup>Universidad Bernardo O'Higgins, Santiago, Chile

### Abstract

Chronic wasting disease is a prion disease affecting cervids captive and free-range. CWD is thought to be caused by indirect exposure to contaminated environments. Many studies have shown that infectious prions can enter the environment through saliva, feces, or urine from infected animals and decaying carcasses. However, we need to understand the specific contribution of this component to disease transmission events. Plants are logical environmental components to be evaluated since they grow in environments contaminated with CWD prions and are relevant for transmission. The main objective of this study is to characterize whether prions are transported to the roots and leaves of carrots, an edible plant commonly used in the human diet and as deer bait. We have grown carrot plants in CWD-infected soils. We harvested the carrots and separated them from the leaves. These materials were interrogated for their

prion seeding activity using the PMCA. Infectivity was evaluated in mouse bioassays (intracerebral injections in Tg1536 mice). The PMCA analysis demonstrated CWD seeding activity in soils contaminated with CWD prions and in carrot plants (leaves and roots) grown on them. Bioassays showed that both plants and roots contained CWD prions sufficiently to induce disease. As expected, animals treated with prion-infected soils developed prion disease at shorter incubation periods (and complete attack rates) compared to plant components. We show that edible plant components can absorb prions from CWD-contaminated soils and transport them to their aerial parts. Our results indicate that edible plants could participate as vectors of CWD transmission.

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## 10 Utilizing RT-QuIC to explore the inhibitory effects of metal ions on CWD prion amyloid formation

Manci Li, Peter Larsen

University of Minnesota, Saint Paul, USA

### Abstract

Real-time quaking-induced conversion (RT-QuIC) is a highly sensitive assay to detect PrPCWD seeding activity in vitro. In addition to assisting future diagnostics of protein misfolding diseases, RT-QuIC has been suggested for drug pre-screening, especially in assessing the ability of compounds to inhibit amyloid formation. Among many candidates for controlling Chronic wasting disease (CWD) in cervids, nutritional additives with metal ions known to interact with PrPC and have enhanced cellular uptake, such as copper amino acid complex (Cu-AA) and zinc amino acid complex (Zn-AA), are ideal candidates to explore potential inhibitory effects on prion amyloid formation. Moreover, such nutritional additives can be effectively delivered through cervid feed. Here, we aim to investigate the potential impact that Cu-AA and Zn-AA have on amyloid formation in vitro using RT-QuIC. We adapted RT-QuIC to characterize the inhibitory effects of Cu-AA and Zn-AA on CWD prion amyloid formation in RT-QuIC and compared such effects with those produced by metal salts alone. We found that Cu-AA and Zn-AA can more readily inhibit amyloid formation in vitro than metal salts and the timing of addition affects the effectiveness of inhibitory effects. Our results suggest that metal ion additives may exhibit inhibitory effects on amyloid formation through interactions with PrPC. Future research efforts aimed at advancing therapeutics for prion diseases using metal complexes are needed.

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## 11 Large-scale PMCA screening of retropharyngeal lymph nodes and in white-tailed deer and comparisons with ELISA and IHC: the Texas CWD study.

Rebeca Benavente<sup>1</sup>, Paulina Soto<sup>1</sup>, Mitch Lockwood<sup>2</sup>, Hunter Reed<sup>2</sup>, Rodrigo Morales<sup>1</sup>

<sup>1</sup>Department of Neurology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, USA. <sup>2</sup>Texas Parks and Wildlife Department, Austin, USA

### Abstract

Chronic wasting disease (CWD) is a prion disease that affects various species of cervids in both free-ranging and captive settings, and has been detected on 3 continents. In Texas, the first case of CWD was detected on Hudspeth county, and now it has been detected in 16 additional counties.

Currently, the gold standard techniques used for CWD screening and detection are ELISA and immunohistochemistry (IHC). Unfortunately, these methods have relatively low diagnostic sensitivity. Two in vitro prion amplification techniques, real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA), have been used to diagnose CWD in several tissues and bodily fluids. Recent studies have shown the potential of RT-QuIC as a rapid and cost-effective method for diagnosing CWD in medial retropharyngeal lymph node (MRPLN) samples. These studies suggest that identification of CWD positive samples is similar for ELISA, IHC and RT-QuIC. In this study, we analyzed 1,003 MRPLNs from both free-ranging and captive white-tailed deer from Texas. These samples were tested by PMCA to assess for the presence of CWD prions, and the results obtained were compared with currently approved techniques. Our results show a 15-fold increase in CWD detection in free-range deer compared with ELISA, and detected CWD in Texas counties with no previous history of the disease. In the case of captive deer, we detected 16% more CWD positive animals when compared with IHC. Interestingly, some of the positive samples displayed differences in their electrophoretic mobilities, suggesting the presence of different prion strains within the State of Texas.

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# 14 Protein misfolding cyclic amplification (PMCA) as an ultra-sensitive technique for the screening of CWD prions in different sample types.

Francisca Bravo-Risi<sup>1,2</sup>, Paulina Soto<sup>1,2</sup>, Rebeca Benavente<sup>1</sup>, Hunter Reed<sup>3</sup>, Mitch Lockwood<sup>3</sup>, Tracy A. Nichols<sup>4</sup>, Rodrigo Morales<sup>1,5</sup>

<sup>1</sup>Department of Neurology, The University of Texas Health Science Center at Houston, Houston, USA. <sup>2</sup>Universidad Bernardo O'Higgins, Doctorado en Ciencias con Mención en Materiales Funcionales, Santiago, Chile. <sup>3</sup>Texas Park and Wildlife Department, Austin, USA. <sup>4</sup>Veterinary Services Cervid Health Program, United States Department of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, USA. <sup>5</sup>Centro Integrativo de Biología y Química Aplicada (CIBQA), Universidad Bernardo O'Higgins, Santiago, Chile

## Abstract

Chronic wasting disease (CWD) is a prion disease that affects farmed and free-ranging cervids. Currently, CWD status is ultimately confirmed in the brain and lymphoid tissues by immunohistochemistry (IHC). One limitation of IHC is its relatively poor sensitivity making it difficult to detect this disease early in the incubation period which can extend 1-3 years. Protein misfolding cyclic amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC) are ultra-sensitive techniques that provide a means to detect CWD in early stages of the disease. PMCA mimics the self-propagation of infectious prions in vitro through multiple incubation-sonication cycles, increasing the number of prion particles present in a given sample. The detection of proteinase K (PK)-resistant PrP<sup>Sc</sup> by PMCA has been performed in experimental and natural samples that may otherwise go undetected using traditional diagnostic techniques.

In this study, we highlight recent advances and contributions that our group has made detecting CWD-prions in animal and environmental samples collected from deer breeding and taxidermy facilities. Additionally, CWD-prions were detected in samples from hunter-harvested, free-ranging animals.

PMCA successfully detected CWD-prions in a diverse array of samples including blood, semen, feces, obex, retropharyngeal lymph node, fetuses (neural and peripheral tissues) and gestational tissues, parasites-insects, plants, compost-soil mixtures, and swabs from trash containers.

Importantly, our findings identified CWD in areas previously considered to be free of CWD. Overall, our findings demonstrate that PMCA is a powerful technique for the screening of biological and environmental samples, and it may prove useful as a CWD management and surveillance tool.

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# 15 Detection of CWD prions in plants collected from white-tailed deer farms

Francisca Bravo-Risi<sup>1,2</sup>, Paulina Soto<sup>1,2</sup>, Yumeng Huang<sup>1</sup>, Tracy A. Nichols<sup>3</sup>, Rodrigo Morales<sup>1,4</sup>

<sup>1</sup>Department of Neurology, The University of Texas Health Science Center at Houston, Houston, USA. <sup>2</sup>Universidad Bernardo O'Higgins, Doctorado en Ciencias con Mención en Materiales Funcionales, Santiago, Chile. <sup>3</sup>Veterinary Services Cervid Health Program, United States Department of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, USA. <sup>4</sup>Centro Integrativo de Biología y Química Aplicada (CIBQA), Universidad Bernardo O'Higgins, Santiago, Chile

## Abstract

Chronic wasting disease (CWD) is a prion disease affecting captive and free-ranging cervids. Transmission of CWD is thought to occur by direct animal-to-animal contact and by exposure to contaminated environmental fomites. CWD-prions are spread into the environment through biological fluids, excreta, and decaying carcasses. Extensive studies demonstrate that soils adsorb infectious prions which can remain attached for extended periods. Accordingly, prion seeding activity has been detected in natural and experimental samples including mineral licks, water sources, and dust. Although, detection of prion in plants under experimental conditions was demonstrated, the role of plants in CWD spreading has been poorly explored. In the present study, we optimized the detection of CWD-prions in plants using the protein misfolding cyclic amplification (PMCA) technology. Specifically, we compared NaPTA pretreatments and direct spiking of the sample into the PMCA reactions. After achieving technical optimizations, we screened multiple plant specimens collected from white-tailed deer breeding facilities displaying variable CWD prevalence. Our results demonstrated that CWD-prion detection for plants was optimal when samples were pre-treated with a NaPTA PrP<sup>Sc</sup> enriching procedure. Our screening results showed positive PMCA activity for specimens collected from the farm with the highest CWD prevalence. Plants from the same site were tested for infectivity in meadow voles, a co-existing animal species that feeds from grass plants. Although meadow voles were highly susceptible to CWD prions by intra-cerebral administration, ingestion of contaminated grass did not induce prion replication in these rodents. These findings further contribute to understand the role of plants in CWD-prion transmission.

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# 17 DETECTION OF CHRONIC WASTING DISEASE PRIONS IN PROCESSED MEATS.

Rebeca Benavente<sup>1</sup>, Francisca Bravo<sup>1,2</sup>, Paulina Soto<sup>1,2</sup>, J. Hunter Reed<sup>3</sup>, Mitch Lockwood<sup>3</sup>, Rodrigo Morales<sup>1,2</sup>

<sup>1</sup>Department of Neurology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, USA.

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## Abstract

The zoonotic potential of chronic wasting disease (CWD) remains unknown. Currently, there are no known natural cases of CWD transmission to humans but increasing evidence suggests that the host range of CWD is not confined only to cervid species. Alarmingly, recent experimental evidence suggests that certain CWD isolates can induce disease in non-human primates. While the CDC strongly recommends determining CWD status in animals prior to consumption, this practice is voluntary. Consequently, it is plausible that a proportion of the cervid meat entering the human food chain may be contaminated with CWD. Of additional concern is that traditional diagnostic techniques used to detect CWD have relatively low sensitivity and are only approved for use in tissues other than those typically ingested by humans. In this study, we analyzed different processed meats derived from a pre-clinical, CWD-positive free-ranging elk. Products tested included filets, sausages, boneless steaks, burgers, ham steaks, seasoned chili meats, and spiced meats. CWD-prion presence in these products were assessed by PMCA using deer and elk substrates. Our results show positive prion detection in all products. To confirm the resilience of CWD-prions to traditional cooking methods, we grilled and boiled the meat products and evaluated them for any remnant PMCA seeding activity. Results confirmed the presence of CWD-prions in these meat products suggesting that infectious particles may still be available to people even after cooking. Our results strongly suggest ongoing human exposure to CWD-prions and raise significant concerns of zoonotic transmission through ingestion of CWD contaminated meat products.

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# 18 Chronic wasting disease (CWD): Transmission, impacts, and management

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## Abstract

With 100% fatality and no cure, chronic wasting disease (CWD) has been steadily spreading worldwide, infecting cervids in 30 states in the United States, four provinces in Canada, three countries in Europe, and South Korea. While numerous studies have been conducted in relation to CWD, the full scope of its negative ecological, social, and economic impacts is not clear. A systematic review of the published peer-reviewed papers (N=134) was conducted to better understand CWD transmission patterns, impacts, and management interventions implemented to limit its spread. The number of CWD-related papers published each year between 2000 and 2022 followed an increasing trend with an average of six publications per year. Most papers were related to CWD prevalence and transmission (39%), human behavior (33%), CWD impacts (31%), and management interventions (16%). Environmental factors such as soil, water, and plants were the most common transmission sources, and a greater CWD prevalence rate was found among adult male cervids. Hunters showed a higher risk perception and were more likely to change hunting behavior due to CWD detection than other stakeholders. Ecological impacts included decreased survival rate, lower population growth, and declining cervid populations. Culling was found to be an effective and widely implemented management strategy to contain prions, although it was associated with public resistance. Despite potentially high negative economic impacts due to CWD, the number of papers in this area was limited. Sustained surveillance, ongoing research, and engagement of affected stakeholders will be essential for future disease control and management.

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## 19 The chronic wasting disease agent from white-tailed deer fails to adapt to sheep upon second passage

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### Abstract

Interspecies transmission of chronic wasting disease (CWD) is highly variable and dependent upon multiple factors. This study used sheep and transgenic mice to investigate the susceptibility of sheep to the CWD agent from white-tailed deer and to characterize subsequent passages of the resulting disease. Suffolk sheep (n=15) with PRNP genotypes VRQ/ARQ, ARQ/ARQ, or ARQ/ARR were inoculated intracranially with CWD prions from white-tailed deer. Western blots and ELISA's were performed on brain and lymphoid tissues to analyze PrP<sup>Sc</sup> accumulation. PrP<sup>Sc</sup> was detected in 4/15 sheep in the brainstem at the level of the obex, with an average incubation period of 41 months. In affected sheep, distribution of PrP<sup>Sc</sup> was limited to the central nervous system, suggesting that environmental shedding of CWD prions from sheep would be negligible. Brain material from one positive sheep (ARQ/ARQ) was used to inoculate mice expressing the mouse (C57BL/6; 20 ul of 10% homogenate) or sheep VRQ (Tg338; 20 ul of 1% homogenate) PRNP. Inoculum from the CWD-positive sheep did not cause disease or result in detectable PrP<sup>Sc</sup> in ovinized mice (800 dpi). However, upon passage to C57BL/6, 4/20 mice were positive with a mean dpi of 684. Upon second passage to C57BL/6 mice, the attack rate increased to 14/15 with a mean incubation period of 380 days. Overall, this data suggests that the CWD agent from white-tailed deer is unlikely to present a major risk to sheep, but further assessments should be conducted in mice expressing the ARQ ovine prion protein.

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## 20 Using real-time quaking-induced conversion (RT-QuIC) to detect prion seeding activity in bobcat (*Lynx rufus*) scat after the consumption of CWD-positive tissue

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### Abstract

Chronic wasting disease (CWD) is a fatal prion disease of cervids that has been identified in North America and Scandinavian countries. Cervids can become infected with CWD through contact with infected animals or through contact with environmental prions, such as those resulting from decomposition of infected carcasses. Scavengers that feed on infected carcasses may either serve as mechanical vectors or sequester or inactivate prions during digestion. The precise role of scavengers in the ecology of CWD remains unknown. In this study, we set out to determine the role of bobcats (*Lynx rufus*) in the ecology of CWD. Three captive adult bobcats were fed dyed meat with scat collected daily until no detectable amounts of dye remained to determine intestinal transit time. Results indicate a transit time of 48 hours. To optimize real-time quaking-induced conversion (RT-QuIC) for detection of prion seeding activity in bobcat scat, we spiked scat with CWD-positive retropharyngeal lymph nodes (RLN) from Rocky Mountain elk (*Cervus canadensis*) and measured seeding activity. Next, we will assess the duration and magnitude of prion persistence in bobcat scat following consumption of CWD-positive tissues. Bobcats (n=3) will be fed ground beef spiked with CWD-positive RLN. The input RLN and fecal material will be analyzed by RT-QuIC to quantify prion seeding activity following consumption. Our findings will determine if bobcats provide an ecosystem service by reducing CWD through consumption or alternatively contribute to CWD spread. More broadly, findings will inform CWD management and set the stage for similar investigations of other consumer species.

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## 22 A Portable, Visible Light-Based Diagnostic Assay for the Detection of Chronic Wasting Disease Prions

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### Abstract

Diagnostic tools for the rapid and reliable detection of prion diseases are limited. Gold nanoparticles (AuNPs) facilitate sensitive and reliable diagnostic techniques via visual color change for the detection of a variety of targets. In parallel, recently developed quaking-induced conversion (QuIC) assays leverage protein amplification and fluorescent signaling for the accurate detection of misfolded prion proteins. Here, we combine AuNP and QuIC technologies for the visual detection of amplified misfolded prion proteins from tissues of wild white-tailed deer infected with chronic wasting disease (CWD). Our newly developed assay, MN-QuIC, enables the detection of misfolded prions by the naked eye. MN-QuIC leverages basic laboratory equipment that is cost-effective and portable, thus facilitating near real-time prion diagnostics across a variety of settings. To assess portability, we deployed to a rural field-station in southeastern Minnesota and tested for CWD on site. We successfully demonstrated that our nanoparticle-based assay is functional in a non-traditional laboratory setting by performing a blinded analysis in the field and correctly identifying all CWD-positive and not-detected (independently confirmed with ELISA/IHC tests) animals at the field site. We conclude that nanoparticle-based assays have great potential for sensitive, field-deployable diagnostics for a variety of protein misfolding diseases.

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## 23 Evaluation of a vaccine for chronic wasting disease based on measurements of serologic responses in captive Rocky Mountain elk (*Cervus canadensis nelsoni*)

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### Abstract

Chronic wasting disease continues to spread across North America and Europe, causing concern for wildlife health professionals and others, as there is no effective treatment; therefore, managers are forced to consider other tools for the mitigation of disease spread such as vaccination.

Our study aimed at quantifying an immune response in captive Rocky Mountain elk (*Cervus canadensis nelsoni*) after being vaccinated with a novel prion vaccine. From August to October 2021, captive elk were grouped in either a control group (n=4), a low dose vaccine group (n=4), or a high dose vaccine group (n=4) and vaccinated. This vaccine series included one initial vaccination, with an additional three boosters per individual. Serum was collected prior to initial vaccination, and prior to each subsequent vaccine administration. Additionally, after the trial was completed and the final booster was administered, serum was collected at weeks 3, 26, and 52. Serum samples were analyzed using competitive ELISAs.

A prion-specific immune response was identified in 7 out of 8 elk who were administered a vaccination in comparison to the control group. A decline in immune response was detected in serum from all vaccinated elk collected 26-weeks post-immunization and remained at similar levels 52-weeks post-immunization. Future work needs to explore alternative vaccine delivery methods and duration of stimulated immune response as well as a controlled challenge study to determine if the immune response elicited by this novel vaccine could act as a preventative tool against chronic wasting disease in cervids.

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## 26 Mechanisms of adaptation of synthetic prions in hamsters

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### Abstract

Prions generated *in vitro* from minimal, non-infectious components (i.e., synthetic prions), cause *bona fide* prion disease in animals expressing syngeneic PrP<sup>C</sup>, albeit with extended, variable incubation periods and incomplete attack rates reminiscent of a species barrier. In contrast, murine synthetic prions (MSP) formed via PMCA with minimal cofactors readily infected and adapted to mice. To investigate if MSP or hamster synthetic prions (HSP) formed under the same conditions are also highly infectious for hamsters, murine WT, hamster WT, or hamster mutant ( $\Delta$ G54,  $\Delta$ G54/M139I, M139I/I205M) synthetic prions were inoculated into Syrian hamsters. All hamsters inoculated with MSP developed clinical signs of prion disease after an extended incubation period whereas none of the HSP-inoculated hamsters developed clinical signs of prion disease. Western blot analysis of brain homogenate from HSP<sup>WT</sup>- and HSP <sup>$\Delta$ G54</sup>-infected hamsters identified protease-resistant PrP<sup>Sc</sup>, indicating subclinical infection. The MSP rapidly adapted to hamsters, with conformational stability remaining stable throughout serial passage, and resulted in emergence of a single strain, suggesting the MSP are comprised of *bona fide* PrP<sup>Sc</sup>. In contrast, HSP<sup>WT</sup> and HSP <sup>$\Delta$ G54</sup> adapted slowly to hamsters, with onset of clinical disease occurring at second passage and conformational stability changing during serial passage. This data suggests the HSP, in contrast to the MSP, are not comprised of *bona fide* PrP<sup>Sc</sup> and instead generate authentic PrP<sup>Sc</sup> through the process of deformed templating. Combined, differences in infectivity between the MSP and HSP in hamsters suggests, under identical formation conditions, the amino acid sequence of PrP dictates generation of authentic PrP<sup>Sc</sup>.

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## 27 Finding the CWD Surveillance Sweet Spot: a balance between agency resources and confidence in disease detection

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### Abstract

Chronic wasting disease (CWD) surveillance requires a significant amount of funding, agency resources, and hunter participation to be effective in detecting and monitoring the disease. Minnesota Department of Natural Resources (MNDNR) has prioritized CWD surveillance plans based on presumed risk factors; these strategies include testing near CWD-positive cervid farms, sampling along state or provincial borders with novel detections, and responding to sick, wild cervids throughout Minnesota. Over the last 7 years (2016-2022), MNDNR has implemented varying CWD fall surveillance strategies using hunter-harvested samples (mean = 10,801 samples per year; range= 3,555-17,658 samples) to calculate apparent prevalence and plan for future management strategies. Metrics to compare surveillance strategies include confidence in detecting the disease, hunter participation/compliance, and the resulting cost of each surveillance. Voluntary sampling efforts were the most costly (\$121-139/sample), failed to reach surveillance goals, but required fewer agency resources (most popular option for agency staff). Voluntary testing resulted in fewer samples due to a decreased hunter participation (3-27% by permit area, 2019) when compared to higher compliance rates under mandatory sampling frameworks (68-104% by permit area, 2022). Mandatory sampling efforts have ranged from requiring testing for all deer across all hunting seasons, to focusing on peak harvest opportunities, such as opening weekend of firearms season. Under mandatory testing requirements, surveillance goals were usually met or exceeded, and costs per sample were reduced (\$72-124/sample); however, nearly twice the number of staff were needed, when compared to voluntary strategies (averaging 388 vs 213 staff, respectively).

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## 28 Serial passages of CWD in cervidized mice fail to increase transmissibility in humanized mice

Qingzhong Kong

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### Abstract

Chronic wasting disease (CWD) is a highly transmissible prion disease in cervids that is widespread in North America and recently found in Europe. Human exposure to CWD has been significant and may increase further over time due to the continuing spread of

CWD, raising serious public health concerns. There is no evidence to suggest large-scale CWD transmission to humans so far. But numerous in vitro prion conversion studies and in vivo transmissibility studies in animal models suggest that the barrier between CWD and humans can be broken and low-level zoonotic CWD infections cannot be excluded. One long-term concern on CWD zoonotic risk is whether the current CWD strains can evolve to become more zoonotic within the cervid species or after adaptation in another species. There has been a report showing that the CWD prion became more potent seeds in converting human prion protein (PrP) substrates in PMCA reactions, suggesting that CWD prions may become more zoonotic after serial passages in cervids.

We tested this hypothesis in vivo using cervidized and humanized transgenic mouse models. We first passaged one CWD isolate each from mule deer and white-tailed deer three times in the cervidized transgenic mouse line Tg12 expressing elk PrP-132M. Then we compared the transmissibility of these CWD isolates, before and after three passages in the Tg12 mice, in humanized transgenic mouse lines expressing human PrP-129M. No transmission in the humanized mice was detected for these CWD isolates either before or after three passages in the Tg12 mice. Our data do not support the notion that simple serial passages of CWD prions in cervids will quickly lead to more zoonotic CWD prions. The reasons for the discrepancy between our in vivo data and the earlier in vitro data will be discussed.

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## 31 Scrapie as the potential origin of chronic wasting disease in white-tailed deer

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### Abstract

White-tailed deer (WTD) are susceptible to the scrapie agent from sheep after oronasal inoculation. However, results from western blotting these brainstems and lymph nodes are difficult to differentiate from WTD infected with chronic wasting disease (CWD). Tissues were examined via enzyme immunoassay (IDEXX), western blot, immunohistochemistry, and bioassay in cervidized mice (Tg12) in order to assess tissue phenotypes upon subsequent passage of the scrapie agent in WTD.

All WTD were euthanized and necropsied following the development of clinical disease and were positive for abnormal prion protein by enzyme immunoassay. Western blotting of retinas from all WTD (second pass) resulted in a similar molecular profile as the retinas of WTD that were inoculated with the agent of scrapie from sheep (first pass). Immunohistochemical staining also was similar between inoculation groups and the initial passage from sheep, but different from WTD inoculated with the agent of CWD. Following bioassays in cervidized mice, all incubation periods were over 300 days, substantially longer than the approximately 200-day incubation period typical with CWD isolates.

Based upon analysis of retinal tissues, it is possible to differentiate the agents of scrapie and CWD in WTD by both western blot and immunohistochemistry. Bioassay in cervidized mice further supports this based on incubation periods of WTD-scrapie being approximately twice that of WTD CWD.

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## 32 Optimal Risk-based Allocation of Disease Surveillance Effort for Clustered Disease Outbreaks: CWD Surveillance in Arizona

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### Abstract

Designing a disease surveillance program to detect a disease is challenging when animals are organized into herds because disease cases are likely to be clustered. Clustered diseases are often surveilled using two-stage sampling, which allocates tests both among herds and within herds. We developed a search algorithm to find the optimal allocation of tests by iteratively adjusting the test allocation for marginal improvements in system sensitivity. We digitally generated 21 herds of various sizes, evenly divided among three regions that differed in relative risk. We then analyzed 29 scenarios that differed in disease and testing characteristics. We also analyzed Chronic Wasting Disease (CWD) surveillance effort for 23 elk game management units of various sizes across three regions in Arizona, USA. We compared our marginal sensitivity approach to two other strategies for approximating the optimal distribution of tests: allocating the same number of tests to all herds selected for testing, and allocating tests so that all herds selected for testing achieve the same sensitivity. Across analysis scenarios, we found that low prevalence, high relative risk, a small budget, or high overhead costs were best addressed by concentrating tests in large, high-risk herds. Across the analyzed scenarios, our marginal sensitivity approach was most efficient, with alternative strategies requiring 0–228 % more tests to achieve the same sensitivity. For CWD in Arizona, we found the potential to double system sensitivity, given a population design prevalence of 0.16 %, from 35.8 % to 70.5 %, although social and budgetary considerations would likely constrain changes to the current allocation of tests. The marginal sensitivity approach we

developed has the potential to improve disease surveillance. An important limitation of our approach is that computer runtimes could become unacceptably long for a population with many herds.

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### **33 Evaluating white-tailed deer harvest in Wisconsin and relationships to management practices and chronic wasting disease**

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#### **Abstract**

For game species, voluntary harvest by hunters is the dominant management and conservation mechanism in the United States. Quantifying the drivers of harvest is critical for determining the limitations of hunter harvest, which can inform management actions. However, it is challenging to quantify the drivers of harvest when analytical applications require data at higher resolutions than is collected. For example, spatial mismatch between hunting regulations, tag issuance, and recorded harvest can be analytically problematic. Additionally, wildlife harvest is comprised of a series of processes, each of which may be limited by different factors. For instance, we could hypothesize that hunters acquire fewer tags in areas with relatively high chronic wasting disease (CWD) prevalence (i.e., “disease avoidance”), but CWD prevalence does not influence the probability of harvesting a deer. Using white-tailed deer in Wisconsin as an example, we separated harvest into its component processes, and analyzed these processes individually. Using Bayesian modeling, we identified the factors influencing the number of tags a hunter acquired, and the probability a hunter harvested a deer (i.e., “filled their tag”). Of particular interest were factors that wildlife managers can manipulate, such as hunting season length, and hunter responses to CWD. This research has implications for human dimensions of wildlife disease management, the efficacy and flexibility of voluntary harvest, and applied wildlife management practices.

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### **34 Disparate tissue effects of pre-analytic differential centrifugation and NaPTA precipitation on enhanced detection of CWD prions by RT-QuIC using RAMALT and MRPLN samples from naturally infected WTD**

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#### **Abstract**

The real-time quaking-induced conversion (RT-QuIC) assay is a type of seeded amplification assay that can provide highly sensitive detection of prions in homogenized tissues, under some conditions detecting prions in homogenates that are many millions-fold more dilute than the harvested tissue. However, detection by amplification is inhibited by constituents of tissue which must be sufficiently diluted (commonly 1,000-fold) before entering the assay. Dilution inherently limits detection during the earliest stages of infection when prion accumulation in tissues is minimal. Several pre-analytic methods of tissue homogenate preparation have been studied that aim to extract prions away from inhibitory tissue constituents and minimize dilution of, or even concentrate, the prions entering the assay. In this study, we use two types of lymphoid tissues from white-tailed deer to compare the standard dilution strategy with the low-cost pre-analytic preparatory methods of differential centrifugation and NaPTA precipitation. The results demonstrate tissue-specific differences in the effects of these methods. Nonetheless, each method when correctly applied improved detection sensitivity compared to the standard homogenate dilution.

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## 38 Human Dimensions of Chronic Wasting Disease: The Calm Before the Storm

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### Abstract

Chronic wasting disease (CWD) represents a significant threat to cervid species. Past experiences in attempting to manage the disease have shown that stakeholder behaviors and the public's trust of agency management actions play a crucial role in effective CWD responses and that even the most sophisticated technical responses to CWD may be derailed by a lack of public support and cooperation. The human dimensions of CWD management were examined in Washington State. While CWD has not yet been detected in Washington, it represents a potentially significant long-term threat to the diverse native cervid species as well as to the human communities who rely on them. Interviews and surveys with stakeholders were used to determine initial public support and opposition for CWD management as well as key perceptions, and overall knowledge. Uncertainties regarding the disease and management, risk perceptions, and issues of trust were all identified as concerns. Groups that may be affected by CWD and CWD management are numerous and encompass hunters, tribal groups, biologists, and rehabilitators among others. Given the wide range of stakeholders, it was found that the viewpoints are notably varied and at times contentious. The importance of applicable education and outreach emerged as pivotal in negotiating the human dimension element of CWD and CWD management, specifically understanding how people are receiving their information and how to improve the dissemination of information. This is essential, as success in managing CWD will increase if a strong human dimensions component is developed and incorporated before an outbreak.

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## 39 Combining vaccination with genetic resistance to protect caribou against CWD

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### Abstract

Our long-term goal is to develop a CWD vaccine to protect caribou and other cervids against CWD. CWD incidence is reaching 15% in mule deer in Alberta hunting areas, a tenfold increase over the last 10 years. Caribou are still free of CWD, but transmission into caribou, a major food source for Northern populations, is likely to happen soon. We described genetic factors in the prion protein (PrP) that likely provide caribou relative resistance to CWD. We propose that vaccination combined with relative genetic resistance creates additive effects. We have established a solid proof-of-concept that vaccination provides protection in CWD challenge models. We recently developed knock-in (KI) mice with different PrP genotypes that recapitulate CWD pathogenesis as found in cervids. Mice and reindeer were immunized as described previously and blood collected for determining humoral and cellular immune responses. Vaccinated and unvaccinated KI mice were infected with reindeer-derived CWD, and vaccine effects on incubation time and prion shedding will be analyzed. The first part of our work analyzes the effect of PrP codon 138 on vaccination followed by CWD challenge in KI mice. This work will also analyze whether vaccination reduces CWD shedding. Vaccination studies are done in parallel in reindeer to test vaccination efficacy in cervids. Additional work will determine effects of oral vaccination, using nanovaccines and vector-based vaccines. CWD challenge studies will be done with white-tailed deer in different settings. Our work will result in tools that help to protect caribou and other cervid species against CWD infection.

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## 41 Building Collaborations to Characterize CWD Risk Relative to Host Aggregation Patterns

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### Abstract

Managers may be able to reduce disease risk through adaptive management of population density or focal concentrations, but the relationship between ungulate distributions and CWD transmission is unknown. We are seeking collaborators interested in ranking relative disease risk across the U.S., quantifying the connection of local density to CWD prevalence and survival, and evaluating the success of adaptive management efforts in areas with appropriate data. This project builds on work conducted at the National Elk Refuge, WY in 2017-2019 evaluating the drivers of elk aggregation and developing a decision support tool assessing methods for quantifying elk densities to aid in mitigating CWD transmission risk. The open-source R package WildAgg allows biologists to input standardized GPS collar or other location data and derive spatially and temporally explicit information to facilitate adaptive management and research across the country. The relative importance of direct versus indirect transmission across the CWD host range is unknown,



yet aggregation patterns influence both in terms of contact rates and time spent in areas where prions are shed. Our goal is to co-design, with collaborators, a multi-population study to better understand the relationship between ungulate distributions and disease transmission. We envision providing support to partners with GPS or imagery data who are interested in characterizing ungulate aggregation patterns (whether or not CWD is currently present in their populations), quantifying the impacts of adaptive management practices and helping to answer specific questions that meet our collaborators' needs.

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## 42 Detecting differences in prion protein conformation by quantifying methionine oxidation.

Christopher J. Silva, Melissa L. Erickson-Beltran

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### Abstract

Cervid PrP<sup>C</sup> contains a greater number of methionines than would be expected for a mammalian protein of its size. Based on a survey of cervid *Prnp* genes, all of the methionines in the mature PrP<sup>C</sup> are contained in a set of seven tryptic peptides: TNMK, MLGSAMSR, LLGSAMSR, ENMYR, MMER, MIER, and VVEQMCITQYQR. The seven peptides are all well-suited for a multiple reaction monitoring (MRM)-based analysis. The chromatographic conditions facilitated a baseline separation of the oxidized and unoxidized peptides. This was true of the unoxidized, two mono-oxidized, and doubly oxidized forms of the MLGSAMSR or MMER peptides.

The thioether of methionine is readily oxidized by added hydrogen peroxide. Thus, hydrogen peroxide can be used to map the surface exposure of cervid PrP methionines by quantitating the extent of methionine oxidation. Recombinant elk PrP (132L or 132M) was oxidized with hydrogen peroxide and then digested with trypsin. The extent that each methionine was oxidized was measured. The results are consistent with the NMR structure of elk rPrP.

This approach can be applied to map the surface of CWD prions. Hydrogen peroxide would be added to purified CWD prions to oxidize methionines based on their surface exposure. The prions would be inactivated and analyzed by mass spectrometry to quantify the extent of each methionine's oxidation, resulting in a map of the surface exposure of methionines in CWD prions. Oxidation by hydrogen peroxide does not result in a significant loss of infectivity, implying that hydrogen peroxide-based oxidation does not perturb the PrP<sup>Sc</sup> conformation.

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## 43 Using aerial surveillance and software to analyze proximity properties of a California tule elk population.

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### Abstract

Aerial surveillance is widely used to survey landscapes and to count animals on those landscapes. California is CWD free. The state manages a herd of native tule elk (*Cervus canadensis nannodes*) that roam freely over an enclosed 2600-acre environment of open grassland and scrub. The USDA/FPAC/GEO aerial surveillance data from this area (Google Earth) was visually inspected for 2013, 2014, and 2022 to identify tule elk (n = 144, 413, and 230, respectively). The orientation of these animals was determined; they did not orient themselves in any particular direction.

The global position system (GPS) locations of the tule elk (2022) were recorded in GPS coordinates. These coordinates were processed by a USGS R package (WildAgg; <https://doi.org/10.5066/P9DTQEJS>). These data were plotted to reveal a digital summary of animals' locations. The software permitted an analysis of the proximity properties of the elk in the 17 observed animal groups.

CWD is believed to cause behavioral changes in infected animals before clinical signs appear. CWD-infected does have fewer fawns, and CWD-infected animals are more susceptible to predation. By using software to identify deer in the wild, they can be enumerated by size and location. In principle, machine learning can be used to compare the distribution patterns of deer residing in CWD-free environments with those of deer residing in environments with varying levels of CWD. Such information can be used to determine if there are observable differences between CWD-free and CWD-infected populations and at what level of CWD prevalence those differences become observable.

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## 44 Evaluating adaptive management to reduce elk aggregations on the National Elk Refuge, WY.

Will Janousek<sup>1</sup>, Eric Cole<sup>2</sup>, Tabitha Graves<sup>3</sup>

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### Abstract

Adaptive management is an iterative process involving learning by doing. In this process management actions are evaluated and adjusted to best achieve management goals. In the United States, wildlife managers are tasked with maintaining populations of culturally and economically important ungulates such as elk (*Cervus canadensis*) in the face of the continued expansion of chronic wasting disease (CWD). To mitigate the threat of disease outbreaks and to meet objectives of sustainable populations of elk on the National Elk Refuge, WY, the U.S. Fish and Wildlife Service established a 'Step-down' management plan to reduce the reliance of elk on supplemental winter feeding which creates unnaturally high aggregation of elk on the Refuge. In this case study, we evaluated six years of data (2017-2022) to determine the efficacy of adaptive management on the Refuge to reduce elk aggregation. We measured changes to elk aggregation using relocation data from GPS-collared elk and a variety of aggregation metrics including kernel density estimates, inter-elk distances, and pairwise interaction via contact rates. We found changes in all metrics indicating reductions in aggregation in years with reduced amounts of feeding on the Refuge. However, metrics varied in sensitivity to changes in aggregation and by time period. These results underscore the complexity of measuring management outcomes and the usefulness of using multiple metrics. We also developed a new R package, 'wildagg,' to streamline the process of calculating the assessment metrics used in this study and to increase the reproducibility of this assessment in the future.

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## 45 Evaluation of residual prions from disinfected prion contaminated stainless-steel surfaces

Vivianne Payne<sup>1,2</sup>, Sara Simmons<sup>1</sup>, Qi Yuan<sup>1</sup>, Jason Bartz<sup>1</sup>

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### Abstract

Prions are abnormal pathogens causing transmissible spongiform encephalopathies (TSEs) or prion diseases, which induce misfolding of normal cellular proteins to pathogenic forms. Iatrogenic and occupational transmission of disease has been documented through incompletely disinfected surgical tools and/or other prion-contaminated sources. Raising concerns about disinfection sufficiency of prion-contaminated environments. A methodology for the evaluation of prion disinfection efficiency is needed. Our group has developed a novel method to detect swab-recovered prions from stainless-steel surfaces that could represent original prion load in terms of seeding activity by real-time quaking-induced conversion (RT-QuIC) assay. This study investigated the performance of the swabbing-RT-QuIC technique, to detect residual surface prions after disinfection. When performing the experiment, circular stainless-steel tokens (grade 304) were contaminated with 50  $\mu$ L of a rodent prion from a 10-fold brain serial dilution. Tokens were left to dry at 22°C overnight before being subjected to undiluted bleach (treatment group) for disinfection or ultrapure water (non-treatment negative control) for 10 minutes. Tokens were then rinsed with ultrapure water before being subjected to swabbing and RT-QuIC. The results indicated a significant reduction ( $\geq 6$  log) in prion seeding activity for bleached tokens. In comparison, the seeding activity from tokens treated with ultrapure water was unchanged ( $< 1$  log). Future experiments will explore the utility of other prion disinfectants, with different levels of effectiveness, with the swabbing-RT-QuIC methodology.

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## 50 Communicating about Chronic Wasting Disease (CWD): Educational Resources for a Variety of Audiences

Marc Schwabenlander, Tiffany Wolf, Peter Larsen, Roxanne Larsen

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### Abstract

Communicating about a complex disease affecting animal and human health can be challenging, especially with the ever-changing dynamic of worldwide connectedness and limited hands-on and virtual resources. There is an increased need for a variety of educational tools related to disease surveillance, food safety, and animal health and welfare. Our goal was to develop resources in

collaboration with partners interested in training and educating several communities impacted by CWD. In collaboration with the Center for Animal Health and Food Safety at the U of MN, an informative animation, booklet, and augmented reality display (Zappar™ App) was developed. We also focused on creating printed and virtual 3D models, since tissues of concern are often difficult to recognize or find during surveillance sampling and/or inspection. Since 2020, two 3D-printed anatomical cervid head models and a companion digital video have been developed. All can be utilized by trainers and educators. Feedback from clients and audiences has allowed us to add new features and refine the models. The development of these CWD educational materials demonstrated a natural progression of engagement and technology, from more basic to increasing complexity. The models and associated materials were used to educate the general public, hunters, cervid farmers, veterinary students, and wildlife biologists in 66 events reaching 30,000 people as of January 2023. Formal evaluation of the utility of these materials has been limited, but subsequent materials and applications will use a framework of educational design to allow for evaluation, assessment, and validation.

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## **53 Chronic Wasting Disease (CWD) Epidemiologic Risk Assessment of Farmed Cervids and Free Ranging Cervids in Kentucky**

Matthew Springer<sup>1</sup>, John Cox<sup>1</sup>, Katie Flynn<sup>2</sup>, [Christine Casey](#)<sup>3</sup>

<sup>1</sup>University of Kentucky, Lexington, USA. <sup>2</sup>Kentucky Department of Agriculture, Frankfort, USA. <sup>3</sup>Kentucky Department of Fish and Wildlife Resources, Frankfort, USA

### **Abstract**

CWD is currently present in six of seven Kentucky-bordering states (Missouri, Illinois, Ohio, West Virginia, Virginia, and Tennessee). The Kentucky Department of Agriculture and the University of Kentucky collaborated on a pilot study to quantify and qualify the current risks associated with interactions between wild cervid populations in the vicinity of farmed cervid herds in Kentucky to provide insights into potential risk factors for CWD introduction and spread. In December 2019 and January 2020, KDA placed cameras on the fence line of four farmed cervid premises for one year to quantify the number and type of free ranging and farmed cervid interactions occurring. Of the three million images collected, 36,974 images of wild white-tailed deer utilizing the areas surrounding the farmed facilities were identified. The majority of these images were animals navigating the landscape surrounding the facilities and did not engage in any type of interactions with farmed animals. A total of 980 images were collected and categorized as close contacts, which were further broken down into about 100 unique instances of wild deer within about 1 meter of the fence line with direct interactions identified. The majority of these close contacts were documented within the breeding season months. Study results recorded direct interactions between wild and farmed deer over varying lengths of time and behaviors. Given the potential for respiratory droplets and/or saliva to be exchanged between individuals within these interactions, it is safe to assume there is some risk of disease spread between farmed and wild cervids.

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## **55 CWD multi-generational mother-to-offspring transmission in Reeves' muntjac deer**

[Erin McNulty](#), Amy Nalls, Edward Hoover, Candace Mathiason

Colorado State University, Fort Collins, USA

### **Abstract**

Chronic wasting disease (CWD) continues to demonstrate geographic expansion. Horizontal transmission is credited for much of the spread of CWD, yet few studies have monitored CWD transmission from mother-to-offspring. We have demonstrated that CWD-infected muntjac dams can become pregnant, carry, deliver, and rear offspring during the long asymptomatic phase of prion infection. Our studies have revealed that CWD prions can be transmitted from mother to first-generation offspring, leading to prion infection and clinical disease progression. To further support gestational CWD transmission we established the presence of infectious prions within the pregnancy microenvironment (uterus, placentomes, amniotic fluids). Here, to determine if CWD mother-to-offspring transmission to a second generation of offspring may contribute to the remarkable transmission efficiency of CWD, we assessed tissues harvested from full-term second-generation nonviable muntjac offspring for infectivity. Transgenic mice expressing the cervid prion protein were inoculated with CWD maternal tissues, tissues from first generation fetuses or tissues from nonviable second-generation muntjac offspring. We found that all mice inoculated with dam tissues, first-generation fetal tissues and fetal tissues from second-generation offspring developed signs consistent with prion disease and were confirmed CWD positive by western blot and RT-QuIC. Mice receiving negative control tissues of similar origin remained free of prion infection. Our data indicates that: (1) multigenerational CWD mother-to-offspring transmission may be possible, and (2) early and persistent exposure of the developing embryo to infectious CWD prions in the uterine microenvironment may help explain the facile transmission of CWD.

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## 57 Increased Sensitivity of RT-QuIC Using Microfiltration

Gage Rowden<sup>1,2</sup>, Mancini Li<sup>1,2</sup>, Marc Schwabenlander<sup>1,2</sup>, Peter Larsen<sup>1,2</sup>

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### Abstract

Real-time quaking-induced conversion (RT-QuIC) has emerged as an exceedingly viable option for the detection of Chronic Wasting Disease within the past decade. The assay's increased sensitivity compared to current antibody-based tests has proven to be an asset to diagnostic programs, yet there are still milestones to overcome to optimize the assay. One such limitation is that tissues must be diluted sufficiently due to inhibitory effects (likely from cell debris) at high-tissue concentrations. This ultimately limits the amount of prions available to seed a given reaction, thus restricting the sensitivity of RT-QuIC. By using a combination of high-speed centrifugation and microfiltration through 0.22µm filters, we show that tissue-related inhibitory effects can be remediated at least 10-fold, allowing detection of prions at concentrations that would otherwise inhibit the reaction. These results are especially critical for testing tissues having very low prion load (e.g., early infection, heterogeneous prion deposition) because normally those tissues would require dilution past the point of detection to prevent inhibition of the RT-QuIC reaction. Given these findings, the method devised here could enhance RT-QuIC sensitivity for the detection of prions in a variety of biological samples.

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## 59 Seasonal variation in white-tailed deer (*Odocoileus virginianus*) habitat selection, encounter locations, and chronic wasting disease transmission potential

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### Abstract

Chronic wasting disease (CWD) can be transmitted between cervids both directly and indirectly through the environment. As such, favored cervid habitats are generally expected to promote direct deer interactions or indirect spatial overlap, and subsequent transmission. However, little is known about how individual animal space use translates to actual sites of spatial or spatio-temporal overlap between individuals. These gaps leave uncertainty in the suitability of habitat selection studies for predicting CWD spatial transmission risk. In this study, we used integrated step selection analysis to quantify seasonal habitat selection for 563 white-tailed deer (WTD) in southwest Wisconsin. We estimated seasonal encounter distributions—regions where a pair of deer were most likely to encounter each other—for all between-group pairs of WTD in our study. We quantified seasonal variation in the habitat composition of each encounter distribution using spatial hurdle models. Preliminary results suggest that WTD favored forested habitat throughout the year, and encounter distributions were primarily composed of these habitats. While agricultural areas like corn fields made up only small amounts of encounter distributions, WTD pairs were significantly more likely to have corn in their encounter distributions in the non-breeding season (winter), as compared to the fawning season (spring). This is despite a pattern of relative avoidance of corn in step selection analyses. These results suggest that habitat selection studies of social species like cervids, by focusing on individual behavior in isolation, can underestimate the importance of habitat types in the context of animal space use and subsequent transmission risk.

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## 61 Development of proactive CWD communication and education strategies through measuring knowledge, behavior, and risk perception of cervid stakeholders in Massachusetts

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### Abstract

Communication around management and prevention of Chronic Wasting Disease has been a consistent challenge for wildlife agencies. With each new state/province that has detected cases, there are new constituencies and stakeholder groups that must be rapidly

educated about the disease and how they can assist with efforts to limit transmission. The extended time it can take for such constituencies to gain competency with disease information can create a communication vacuum which may be filled with misinformation. In order to develop proactive CWD communication and education strategies we administered online surveys to several cervid stakeholder groups within Massachusetts including 40,000 hunters, all Captive Cervid Permit holders, MassWildlife staff, and the Massachusetts Environmental Police officers. CWD has not been detected previously in any cervid species within Massachusetts or New England. The nearest wild deer populations with recent cases are in Pennsylvania, so most resident hunters' knowledge of CWD comes from out of state resources. Based on 8,649 survey responses, we found hunting license holders to lack most basic knowledge about CWD and its transmission pathways with the majority of responses to 16 of 23 knowledge questions to either be incorrect or that they did not know the answer. The survey results indicated a likely receptive population with 88% of respondents stating that the prevention of CWD introduction was moderately or highly important to them. Based on the results of the surveys, specific communication channels have been targeted for education to address knowledge gaps across each stakeholder group.

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## 62 Detecting Potential Mycoremediation of CWD Prions in Fungi by RT-QuIC

Rachel Shoemaker<sup>1,2</sup>, Suzanne Stone<sup>1,2</sup>, Gage Rowden<sup>1,2</sup>, Peter Christenson<sup>1,3</sup>, Sarah Gresch<sup>1,2</sup>, Mancu Li<sup>1,2</sup>, Marc Schwabenlander<sup>1,2</sup>, Tiffany Wolf<sup>1,4</sup>, Sang-Hyun Oh<sup>1,3</sup>, Peter Larsen<sup>1,2</sup>

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### Abstract

Chronic Wasting Disease (CWD) continues to spread across three continents, threatening cervid health and ecosystems through prion deposition. Fungi have an intrinsic mycoremediation mechanism for heavy metals, which have a high affinity to prions. Since white-tailed deer (WTD) browse a variety of mushrooms, it is possible that CWD can be transmitted through contaminated fungi. Attention has focused on plant uptake of CWD prions (PrP<sup>CWD</sup>); however, the potential of fungi as PrP<sup>CWD</sup> reservoirs has not been explored. We established an ecological research site in northern Minnesota that is consequent to illegal dumping of CWD-positive WTD carcasses. Various fungi have been observed in close proximity to these remains, leading us to question the role of fungi in PrP<sup>CWD</sup> bioabsorption. Here we investigate the prion uptake potential for *Pleurotus ostreatus*, the common oyster mushroom, within a laboratory setting. Methods consisted of three growth conditions for ~40-60 days, controlling for environmental variables in the absence of WTD brain homogenate as well as the presence of CWD-negative and CWD-positive brain homogenates. We initially observed false seeding activity and inhibitors in normal and negative conditions. After refining the RT-QuIC protocol to overcome the fungal-associated barriers, we observed seeding only in mushrooms grown in CWD-positive WTD brain homogenate. Future research includes RT-QuIC surveillance of fungal specimens collected from the CWD-positive research site using our fungal protocol. These initial data indicate the possibility of PrP<sup>CWD</sup> fungal bioabsorption and the potential role of mushrooms in the transmission of CWD.

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## 63 Fast Axonal Transport of PrP<sup>Sc</sup>

Sam Koshy, Ronald Shikiya, Anthony Kincaid, Jason Bartz

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### Abstract

Peripherally acquired prion diseases such as CWD invade the CNS via defined neuroanatomical pathways that involve the lymphoreticular system, autonomic nervous system, and spinal cord. Transport of disease-causing prions (PrP<sup>Sc</sup>) has been approximated as slow axonal transport, but the methods utilized in these studies have low sensitivity and measure both inoculum PrP<sup>Sc</sup> and newly replicated PrP<sup>Sc</sup>, confounding the observed rates. To more accurately measure PrP<sup>Sc</sup> transport, we utilized highly sensitive protein misfolding cyclic amplification (PMCA) to measure hamster PrP<sup>Sc</sup> transport in the mouse sciatic nerve (ScN), a hamster prion replication deficient system. We also purified and fluorescently labeled PrP<sup>Sc</sup> and imaged particles in live mouse ScN explants to directly measure and record PrP<sup>Sc</sup> transport using two photon confocal microscopy. After PMCA, we failed to detect hamster PrP<sup>Sc</sup> in uninoculated mouse sciatic nerve (ScN) 24 hours (post infection) p.i. but PrP<sup>Sc</sup> was detected in the inoculated ScN and lumbar spinal cord (SC) 24 hours p.i. Based on the distance from the inoculation point to the lumbar SC, PrP<sup>Sc</sup> transport rate was calculated as at least 25 mm/day, well above established slow transport rates (0.3-8 mm/day). Fluorophore conjugated PrP<sup>Sc</sup> was successfully imaged and tracked in live ScN explants, and multiple timeseries were recorded on multiple axonal segments throughout the ScN. Analysis of PrP<sup>Sc</sup> recordings using the ImageJ plugin Trackmate, and recorded velocity rates were consistent with fast axonal transport. These data suggest that PrP<sup>Sc</sup> can use a fast axonal transport mechanism, having implications for peripherally acquired PrP<sup>Sc</sup> involved in CWD.

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# 68 Using a spatially-explicit agent-based disease spread model to identify high risk areas and most cost-effective control strategies in the scenario of potential introduction of Chronic Wasting Disease in California

Carlos Gonzalez-Crespo<sup>1</sup>, Cesar Herraiz<sup>2</sup>, Brandon Munk<sup>3</sup>, Leila Knoll<sup>1</sup>, Alexander Heeren<sup>3</sup>, Linell Hansen<sup>3</sup>, Beatriz Martinez-Lopez<sup>1</sup>

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## Abstract

Chronic wasting disease (CWD) is a fatal, neurodegenerative prion disease of cervids. In North America, it affects white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), moose (*Alces alces shirasi*), and reindeer/caribou (*Rangifer tarandus*). The disease spreads through direct contact with infected individuals or indirect contact with infectious material (e.g., fluids, feces, and tissues of infected animals). First recognized in Colorado in 1967, CWD has incessantly spread across North America having been detected in captive or free-ranging cervids in at least 30 states and 4 Canadian provinces. When unmanaged in free-ranging populations, CWD expands geographically and prevalence increases, lowering adult survival rates and destabilizing population dynamics. While CWD has never been detected in California, despite varying levels of surveillance since 1999, CWD remains a constant threat and could be introduced in California through the natural or anthropogenic movement of infectious animals or materials. By incorporating real mule deer population data, this study presents a 10-year epidemiological simulation of the expansion of a theoretical CWD introduction in California, using an agent-based model approach. Several scenarios were modeled based on different deer densities and proposed management measures (e.g., surveillance for initial detection and initial response, targeted culling, harvest and carcass management). The model provides an estimate of the effectiveness of the proposed management strategies and quantifies the probability of CWD infection across different California deer herds, effects of direct and indirect transmission events, and effects on populations dynamics under diverse disease introduction scenarios. The aim is to provide a valuable tool to support the allocation of risk-based surveillance to prevent, detect and control CWD in the case of a potential introduction in California.

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# 69 Exploratory use of RT-QulC to quantify and compare kinetics of amyloid formation in North American and Scandinavian CWD prions

Leo Tyer<sup>1</sup>, Xutong Shi<sup>1</sup>, Juliana Sun<sup>1</sup>, Sehun Kim<sup>1</sup>, Sylvie Benestad<sup>2</sup>, Glenn Telling<sup>1</sup>

<sup>1</sup>Colorado State University, Fort Collins, USA. <sup>2</sup>Norwegian Veterinary Institute, Oslo, Norway

## Abstract

Chronic wasting disease (CWD) is a transmissible, universally fatal neurodegenerative disease caused by the pathogenic misfolding of prion protein. CWD is endemic to North America (NA), and most recently has been identified in Scandinavian cervids. Interestingly, isolates collected from Scandinavian CWD cases have been shown to possess unique strain characteristics. Previous research from our group has shown Scandinavian CWD is etiologically distinct from its North American counterpart, which may account for these strain differences.

Here, we seek to further characterize the kinetic properties of Norwegian CWD (NorCWD) isolates through real-time quaking-induced conversion (RT-QulC) assay, a method for quantifying the rate a prion seed can form amyloid from a standard substrate. Our preliminary results show that Nor red deer and moose isolates display variable amyloid formation rates within technical replicates, with little discernible linear range over a dilution series. This is distinct from NA elk and mule deer, which display a predictable linear range over 10<sup>-4</sup> to 10<sup>-7</sup> titrations. Notably, the NorCWD isolates converted substrate at a faster rate than seeds taken from NA. Taken together, these results indicate that the distinct properties of NorCWD can be recapitulated using a standard in vitro assay with recombinant Syrian hamster PrP as a substrate. These preliminary data will guide the direction of our future research investigating features influencing NorCWD pathogenicity.

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## 70 Risky Business: Relating Probability of Direct Contact with Risk of Chronic Wasting Disease

Maria Dobbin<sup>1</sup>, Peter Smolko<sup>2</sup>, Laurens Put<sup>1</sup>, [Evelyn Merrill](#)<sup>1</sup>

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### Abstract

Chronic wasting disease (CWD) is a fatal, prion disease of cervids that was first detected in Alberta in 2005. Transmission of CWD occurs by direct contact with infected individuals and via contaminated environments. We investigate the seasonal effects of grouping patterns and landscape heterogeneity on direct, pair-wise contacts within and between sex-specific (same or mixed-sex) groups of mule deer (*Odocoileus hemionus*) in central eastern Alberta. First, we establish criteria based on spatial-temporal movements of collared deer to define sex-specific group membership. Second, we model the relative risk of sex and group-specific contacts occurring in a locale based on landscape characteristics. Third, we relate seasonal predictions of the spatial contact probabilities to the risk of deer being CWD-infected in an area based on hunter-harvest, CWD surveillance data. We determined that in winter contacts occurred in areas with higher use by deer, whereas in summer, contact locations were less constrained and were more varied between sexes. Relative contact probabilities of within and between-group male dyads in winter and between-group female dyads in summer were the best predictors of CWD risk in east-central Alberta. Our results relate habitat specific, social behaviours between conspecific mule deer to potential routes of CWD transmission and contribute to CWD research that guides management strategies for an emergent wildlife disease.

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## 72 Chronic Wasting Disease Complicates Traditional Survival in in Northwestern Arkansas

[Marcelo Jorge](#)<sup>1</sup>, Lisa Jorge<sup>1</sup>, Dana Jarosinski<sup>1</sup>, Mark Ruder<sup>1,2</sup>, Michael Chamberlain<sup>1</sup>, Gino D'Angelo<sup>1</sup>, Richard Chandler<sup>1</sup>

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### Abstract

Chronic Wasting Disease (CWD) is a fatal prion disease that is contagious to captive and wild cervids. As CWD has been discovered throughout the United States and Internationally, concern has grown over the long-term viability of cervid populations within CWD endemic regions. Some studies have modeled population declines in white-tailed deer, mule deer and elk within CWD endemic areas, but most of these studies have occurred in areas where CWD has been present for decades. We investigated survival probabilities of CWD positive and presumed negative white-tailed deer within Arkansas' CWD management zone where CWD was first detected in 2016. Deer were captured and affixed with GPS collars and rectoanal mucosa-associated lymphoid tissues (RAMALT) were collected for testing. If a mortality occurred, the obex region of the brain stem and retropharyngeal lymph nodes were also collected and tested. Sample CWD prevalence was high for a relatively recently detected population. We used a parametric survival model with time-varying covariates to investigate the factors that impacted survival. To date, survival has been lower than in most studies for white-tailed deer from CWD endemic areas so little time after first detecting it. Daily terrain ruggedness index, daily terrain roughness, and daily temperature were positively correlated with survival. Deer that tested positive for CWD had lower survival than those that tested negative. Our results suggest that CWD is reducing annual survival, and CWD may be working in concert with environmental factors (e.g., landscape structure and weather) and other diseases to reduce the long-term viability of cervid populations.

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## 74 Reducing Potential Environmental Chronic Wasting Disease Transmission at Livestock Salt and Mineral Licks

[Bevin McCormick](#)<sup>1</sup>, Paul Cross<sup>2</sup>, Jennifer Malmberg<sup>1,3</sup>, Justin Binfet<sup>4</sup>, Erik Maichak<sup>5</sup>, Cheyenne Stewart<sup>5</sup>, Kevin Monteith<sup>1</sup>, Rhiannon Jakopak<sup>1</sup>, Brandon Werner<sup>4</sup>, Brant Schumaker<sup>1</sup>

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### Abstract

With rapid spread of chronic wasting disease (CWD) throughout North America and Scandinavia in the last two decades, identifying potential hot spots for direct and environmental transmission of CWD is important to cervid conservation. Mineral licks have been shown to attract wildlife and to likely serve as environmental reservoirs for CWD. Furthermore, environmental factors such as high clay content in soil, like that found in regions of Wyoming, USA, may increase transmissibility of prions. The objectives of our study were to

assess the potential impact of livestock salt and mineral licks on CWD transmission and to test devices that may limit cervid access and visitation, while allowing cattle free access to mineral resources. We tested on-the-market cattle mineral feeders as excluding devices at forty-nine sites in three geographical regions in Wyoming. We reviewed game camera images and mule deer GPS collar data to assess the use of salt and mineral lick sites by mule deer, elk, and moose before and after deployment of excluding devices. Preliminary results from a small sample of sites that were treated with mineral feeders in 2021 show that excluding devices prevented access by *Odocoileus* spp in 98.4% of observations and moose in 100% of observations and allowed livestock access. Additional preliminary analysis from sites that were open in 2021 and treated in 2022 showed reduced visitation time of elk by 99.2% and mule deer by 66.9%. While visitation time decreased, elk accessed contents in all regions where elk were present. Our findings indicate that the use of on-the-market feeders can reduce cervid access and visitation at livestock salt and mineral resources, providing a potential management technique that could reduce CWD transmission where livestock occur.

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## 75 Time and CWD marches on in Alberta

Margo Pybus<sup>1,2</sup>, Anne Hubbs<sup>3</sup>, Mark Ball<sup>1</sup>, Peter Smolko<sup>4</sup>, Evelyn Merrill<sup>2</sup>

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### Abstract

Alberta has wrestled with CWD since 1998 and surveillance of hunter-killed cervids was continuous ever since. Circa 2000, CWD entered the province from adjacent Saskatchewan through natural movements of wild deer along two primary watersheds. Active surveillance revealed the first case in a wild deer in Alberta in 2005. The pattern of disease occurrence changed over time. Initially, CWD had a limited distribution in small local areas and groups of deer, particularly mule deer. Slowly, CWD spread westward along major rivers, and travelled up secondary valleys and tributaries. In enzootic areas, as local prevalence increased, focal areas of disease coalesced and CWD became pervasive across the broader landscape. Currently, CWD extends into the east slopes of the Rockies in southwestern Alberta, is widespread throughout parkland and prairie regions of central and eastern Alberta, and borders the southern fringe of the northern boreal forest. Ongoing surveillance data used in multiple research efforts revealed consistent patterns. Generalized Additive Models that included spatial and temporal processes of disease spread indicated prevalence of CWD was higher in mule deer than white-tailed deer and for males than for females. Prevalence was most heavily influenced by the forest cover, river and streams, terrain ruggedness and distance to large cities. We projected spatial patterns of spread indicating when key areas of deer ranges are expected to have prevalence levels > 0.25.

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## 76 Inferring disease status from movement behavior: GPS data alone accurately predict infection with chronic wasting disease in mule deer

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### Abstract

Increased surveillance of wildlife diseases is needed to better understand the epidemiology of emerging pathogens, assess transmission risk, and develop control measures. Yet, wildlife health investigations remain challenging, with costs associated with field collection, transportation of samples, molecular analysis, and interpretation of results. The development of novel diagnostic tools therefore will be critical to better manage emerging diseases. Chronic wasting disease (CWD), a fatal prion disease affecting deer species across North America, can alter normal movement behavior in hosts. Here we present a novel diagnostic method wherein we use GPS collar data from free-ranging animals to infer infection status with CWD. Using two distinct herds of mule deer (*Odocoileus hemionus*) in central Wyoming ( $n = 179$  individuals), we employed an artificial intelligence modeling technique to compare the spatial behavior of CWD-positive deer to those in which CWD was not detected. CWD-positive deer moved slower and used areas closer to water sources compared with deer in which CWD was not detected. Notably, our model was able to differentiate between disease-free individuals and those with clinical stages of CWD with high accuracy. We will present our full predictive model, including cross-validations to movement data of animals from different herds, accounting for age, sex, and malnourishment. We also will demonstrate model applications, such as identifying habitats prone to environmental contamination. CWD is a significant management concern in North America. Our modeling approach directly informs disease prevalence and spread, which ultimately could help target proactive



management of deer species in areas affected by CWD.

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## 77 Using ante-mortem real-time-quaking-induced-conversion to better understand CWD prevalence in the Wind River Basin

Tucker Russell<sup>1</sup>, Brain Dugovich<sup>2</sup>, Emma Tomaszewski<sup>2</sup>, Darly Lutz<sup>3</sup>, Jon Desonier<sup>3</sup>, Samantha Allen<sup>4</sup>, William Edwards<sup>4</sup>, Patrick Hnilicka<sup>5</sup>, Glen Sargeant<sup>6</sup>, Paul Cross<sup>7</sup>, Jerod Merkle<sup>1</sup>, Jennifer Malmberg<sup>8,9</sup>

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### Abstract

Prevalence of chronic wasting disease (CWD) varies widely across herd units and cervid species in Wyoming, USA. The Wind River Basin, located in central Wyoming, is an area of high interest as mule deer within the region experience relatively high CWD prevalence rates, exceeding 60% in adult bucks. This area is characterized by an array of agricultural, anthropogenic, and natural landscapes, as well as multiple jurisdictions including private, state, federal, and tribal land. Therefore, management of CWD in this area is multifaceted, and an assessment of the drivers of high CWD prevalence within the region is a high priority. In this study, we will collect ear punch biopsies from captured mule deer for ante-mortem CWD assessment via real-time quaking-induced conversion (RT-QuIC). To optimize the RT-QuIC assay, we used ear punch biopsies from hunter harvested mule deer previously tested by ELISA. CWD status, determined from ear punch biopsies obtained at capture, will be assessed in conjunction with movement data from mule deer fitted with GPS collars. We will compare habitat and land use as well as movement strategy (e.g., resident vs. migrant) between mule deer that test positive versus negative. Doing so will help identify habitats that facilitate indirect and direct transmission, both of which could be important for maintaining high CWD prevalence in this region. This research will better inform management practices within the Wind River Basin by providing information on an ante-mortem CWD test and identifying potential hotspots for CWD transmission that could be targeted for intervention.

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## 78 Local Resident Perspectives on Chronic Wasting Disease in Tennessee

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### Abstract

Best management practices (BMP) to prevent the spread of chronic wasting disease (CWD) include herd management, or population reduction; however, little is known about the acceptability of this controversial method to local residents and landowners. This study used a mail survey of residents in CWD impacted counties in West Tennessee to assess their willingness to engage in various BMPs to help prevent CWD spread on private lands, their perceptions regarding the effectiveness of herd reduction as a CWD management strategy, and willingness to allow targeted removal of deer from their property. We found a low proportion of respondents believed that herd reduction relying entirely on sport hunting would be effective and fewer than half indicated they were likely to adopt five of the six CWD BMPs for landowners presented. Although a majority of respondents were willing to allow TWRA to conduct CWD research activities on their land, few were willing to allow hunters on their property. About 40% of those opposed indicated they would reconsider provided evidence of the effectiveness of targeted removal on private lands in other areas/states or assurance they would not be responsible for any costs. As responsible agencies formulate regional strategies to contain the spread of CWD, findings from this study can inform the development of specific interventions and associated messaging to cultivate buy in on the part private landowners, a key stakeholder group, in CWD affected areas.

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## 82 Designing an immunoassay with an increased sensitivity for the detection of Chronic Wasting Disease prion

Joseph Ramos, Gillian Castro, Veronica Hafner, Shigetoshi Eda

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### Abstract

Chronic wasting disease (CWD) is a neurodegenerative condition that primarily affects cervids. It is caused by infectious prions that can be transmitted through animal-to-animal contact, or a contaminated environment. The Tennessee Wildlife Federation estimates that the disease would cost the state \$98 million in economic activity and 1,400 jobs, while the direct cost to the Tennessee Wildlife Resource Agency in 2020 totals \$1.8 million. The control and prevention of CWD, along with adequate management techniques, are important to mitigate the impacts of the disease. However, current methods for CWD detection lack sensitivity for testing live animal samples or suffer long turnaround time for results. To meet this need, we designed a new assay capable of detecting CWD prion in tissue samples of harvested animals within one day, and with a sensitivity that may enable screening of antemortem sources. The new assay, named Enzyme Dependent Apta-Sensor Nanoparticle Surface Enriching Immunoassay (EDA-SeNSEI), combines magnetic beads, functionalized gold nanoparticles, and polymerized horseradish peroxidase to concentrate prion protein (PrP) and increase the number of peroxidase molecules per PrP. Using recombinant bovine cellular PrP (PrP<sup>C</sup>) as a model, EDA-SeNSEI demonstrated increased sensitivity compared to a previous version of our assay which distinguishes CWD-positive and negative animals with 100% accuracy using obex homogenates samples.

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## 83 Blood serum-derived extracellular vesicles as a potential peripheralization mechanism for Chronic Wasting Disease in White-tailed deer

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### Abstract

Prions have been detected in the blood of chronic wasting disease (CWD)-infected cervids. How or if prionemia impacts pathogenesis and disease progression has not been well studied. Our current investigations include analysis of extracellular vesicles (EVs) for their role in prion peripheralization. EVs are nano-sized vesicles (30-150nm) known to be released from virtually all cell types and have been demonstrated to facilitate intercellular communication via transport of RNA, lipids, and proteins between cells. It has been reported that EVs facilitate dissemination of both forms of the prion protein, (cellular (PrP<sup>C</sup>) and disease-associated (PrP<sup>Sc</sup>)) in other prion diseases. To begin to unravel mechanisms associated with CWD peripheralization in the host, we are investigating the role of blood serum-derived EVs as PrP<sup>C</sup> and PrP<sup>Sc</sup> transport carriers. Our preliminary studies have resulted in EV isolation from blood serum collected from naïve and experimental CWD-infected white-tailed deer (WTD) and transgenic cervid prnp expressing mice. Nanoparticle tracking analysis (NTA) was used to quantify the size distribution and concentration of the EV isolates. Western blot and real-time quaking induced conversion (RT-QuIC) were used to verify the presence of EVs and prion seeding activity, respectively. Our preliminary studies have revealed that, in our hands, EVs can successfully be isolated from WTD and murine blood serum. Verification of EV-specific markers and prion seeding activity are ongoing. These studies will determine the role EVs may play in CWD peripheralization and elucidate transmission mechanisms from mother-to-offspring.

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## 84 Examining sensitivity levels of high throughput RT-QulC methods for CWD diagnostics

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### Abstract

Real-time quaking-induced conversion (RT-QulC) has emerged as a promising tool for prion research and has diagnostic capacity for various prion diseases, especially CWD. Testing demand is increasing for biological and environmental CWD diagnosis, making high-throughput testing an attractive option. Using 384-well plates can provide up to a 5-fold increase in testing output compared to 96-well plates. To determine if the change in plate format impacts diagnostic sensitivity and specificity, we compared RT-QulC results generated by the two plate types. We used 35 samples of various tissue types (lymph node, brain, muscle) and two controls (lymph node), including a serially diluted positive control, keeping other assay parameters consistent. All samples had been previously characterized by ELISA and IHC. The overall concordance between plates was good (Kappa coefficient: 0.69, 95%CI: 0.49-0.88). The agreement was improved to very good (Kappa coefficient: 0.88, 95%CI: 0.73-1.0) when muscle samples were excluded from the analysis, which showed to have the lowest concordance among tissues tested (0.29, 95%CI -0.11-0.7). Two of the eight muscle samples, which were statistically positive (P-value: <0.005 one-way ANOVA of the max-point ratio versus the negative control) on the 96-well plate, were missed (statistically negative) on the 384-well plate. False negative muscle samples associated with 384-well plates reveal a need to optimize RT-QulC methods using high-throughput formats.

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## 85 Evaluating the Statewide Expansion of CWD Sampling in Minnesota

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### Abstract

Two recent detections of chronic wasting disease (CWD) in Minnesota (Climax, October 2021; Grand Rapids, February 2022) were in areas of the state that were not receiving targeted surveillance based on elevated risk factors as defined in Minnesota's CWD Response Plan. In response to these new detections and increased public interest across the state, the Minnesota Department of Natural Resources (MNDNR) developed statewide sampling programs for the first time in its history by expanding its network of sampling partners (mostly taxidermists) and implementing a new mail-in sampling program. These statewide programs allowed any hunter to have their deer tested for CWD at no charge. Across the state, MNDNR recruited 162 sampling partners and distributed 5000 mail-in kits during fall 2022. This statewide sampling program yielded 3,652 samples and 6 CWD positives (28% of total fall samples, 23% of fall CWD positive samples). Of the samples received, 44% (n=1,598) came from outside active 2022 CWD surveillance areas; these statewide programs did not lead to any detections in new areas. The approximate cost (USD) per successful sample for both the partner sampling program and the mail-in sampling program (\$53/sample and \$60/sample respectively) in 2022 are lower than those associated with mandatory surveillance in 2021 (\$106/sample). Mandatory surveillance requires staff to be present in the field whereas these program do not. While this appears to be successful from a hunter-service perspective, the uneven sample distribution and low quantities across the state did not yield meaningful disease prevalence information.

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## 87 Linking Antler Point Restrictions to CWD Spread and Growth: An Agent-Based Modeling Approach with Empirical Data

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### Abstract

Wildlife management agencies have limited information on how deer harvest regulations affect the composition of the true state of deer populations. This limitation is relevant because chronic wasting disease (CWD) transmission rates are related to sex and age composition, with males playing a critical role in disease dynamics. Thus, different harvest regulations could have different implications for CWD transmission. In 2019, Michigan's Natural Resource Commission implemented mandatory antler point restrictions (APRs) in the state's CWD Core Area, and a study was designed to estimate subsequent impacts on white-tailed deer population abundance by

sex and age class. We used these empirical parameter estimates with varying regulation treatment to inform an agent-based model to simulate and investigate CWD spread and growth. Our objectives were to evaluate how the male segment of deer populations may influence CWD dynamics in Michigan landscapes. We implemented a Michigan-specific, agent-based modeling framework to link deer demography with CWD transmission dynamics. This framework included a population-simulation model that incorporated geographic data, demography, and dynamic social structure to produce population snapshots. These snapshots were used to initialize a spatially-explicit disease model to simulate CWD transmission across deer contact networks. We found that composition of males played an important role in CWD dynamics, and consequently CWD dynamics varied by harvest treatment. By evaluating population parameter information relative to a harvest manipulation and incorporating this information directly into a simulation model developed to investigate CWD dynamics, we help inform the connection between harvest regulations and CWD epidemiology.

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## **88 Investigating chronic wasting disease transmission hotspots and immunity tradeoffs in mule deer in the Wind River Basin**

Brian Dugovich<sup>1</sup>, Tucker Russell<sup>2</sup>, Emma Tomaszewski<sup>1</sup>, Daryl Lutz<sup>3</sup>, Zach Gregory<sup>3</sup>, Jon Desonier<sup>3</sup>, Samantha Allen<sup>4</sup>, Patrick Hnilicka<sup>5</sup>, Glen Sargeant<sup>6</sup>, Jerod Merkle<sup>2</sup>, Jennifer Malmberg<sup>2,7</sup>, Paul Cross<sup>1</sup>

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### **Abstract**

Chronic wasting disease (CWD) persists as an enzootic in southeastern Wyoming. Surveillance has revealed expansion into the Wind River Basin with mule deer prevalence reaching 60% by 2019. An unidentified risk factor such as an environmental transmission hotspot may underlie the region's high prevalence. Potential management targets to help mitigate CWD environmental transmission include agricultural fields, baiting, mineral/salt licks, and carcass disposal sites. However, traditional coarse-scale spatial ecological methods are insensitive to these subtle, fine-scale hypothetical hotspots. Our primary objective is to identify focal or subpopulation hotspots of transmission in the Wind River Basin by combining movement, density, and disease data at refined spatial scales. Additionally, we are examining CWD ecoimmunology as our secondary objective. Despite the causative prion of CWD being lymphotropic, the interactions of the immune system, CWD, and the environment are often overlooked, because prion infection does not induce the classical antibody response. However, prion diseases utilize the immune system for invasion and replication. Thus, a tradeoff between immune responses and CWD progression may exist where animals that are genetically predisposed to longer CWD incubation may exhibit poorer overall immunity. We aim to assess the immune response with blood-based immunoassays in the ecological context of the greater spatial study. We hope to utilize this unique study system to better understand CWD environmental transmission and immunopathology that would provide a useful context for management actions.

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## **89 Large-scale assessment of spatial genetic structure to assess risk of white-tailed deer populations to chronic wasting disease**

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### **Abstract**

Chronic wasting disease can be spread by direct and indirect transmission, but most research focuses on direct transmission or movement of disease between subpopulations. Identifying subpopulations of deer across regions allows delineation of focal areas to target for effective intervention. Our aim was to assess population structure of white-tailed deer (*Odocoileus virginianus*) in the northeastern United States at a larger scale than previously studied and evaluate whether this information can be used to improve resolution of assignment tests. Ten microsatellites were used for 5701 samples collected from wild deer in Maryland, New York, Ohio, Pennsylvania, and Virginia from 2014 to 2022. We used programs STRUCTURE and GeneLand to assess subpopulation structure by partitioning genotype frequencies in subsamples of 2 deer per sex per county. We used this subsample to create simulated populations of 500 individuals for each genetically distinct cluster as well as physiographic provinces and management units to perform genetic assignment tests. STRUCTURE results showed that there were only 2-3 clusters across the region, while GeneLand indicated the presence of 8 clusters. Genetic assignment tests provided further understanding of subpopulation structuring depending on the analysis used and whether location-specific details were included for each genetic sample. The potential for delineation of white-tailed deer subpopulation based on coarse genetic distinction is important to consider when managing for chronic wasting disease. Future

research on what landscape barriers may lead to these divisions and single-nucleotide polymorphisms developed for white-tailed deer may further our understanding of potential subpopulations in the region.

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## 93 Estimating Disease Risk by Integrating Animal Movement into Mathematical Models

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### Abstract

Wintering elk densities on the National Elk Refuge (NER) reach up to ~1,100 elk per km<sup>2</sup>, raising concerns about disease transmission. Increased aggregation and contact rates due to supplemental feeding has been documented, but how those behavioral changes translate into disease risk on the landscape is unclear, and likely depend on the details of disease transmission (e.g., elk-to-elk versus indirectly through soil). Here we aim to answer: how does the practice of supplemental feeding alter the distribution of disease risk across a landscape and does that distribution change under different disease transmission scenarios? Using global positioning system collar data from elk on the NER between 2017 and 2019, we compare the spatial distribution of the force of infection during winters with supplemental feeding (2017, 2019) and without (2018) using a modelling framework, MoveSTIR, that integrates animal movement with a mathematical model of disease to quantify risk across time and space. We find that years with supplemental feeding and harsh winters had higher overall disease risk (quantified by  $R_0$ ) and more concentrated risk in fewer and more high-risk areas, but that the spatial distribution of risk varies with the characteristics of disease transmission.

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## 94 Preliminary results for neurofilament light chain as a serum biomarker for chronic wasting disease in white-tailed deer

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### Abstract

Serum neurofilament light chain (NfL) is elevated in neurodegenerative diseases including human prion diseases and sheep with clinical scrapie. The aim of this study was to evaluate serum concentrations of NfL in white-tailed deer (WTD) with chronic wasting disease. Ten WTD with the wild-type prion protein genotype were naso-orally inoculated with 1 mL of a 10% w/v brain homogenate (0.1 gram) from a WTD with chronic wasting disease (CWD). Serum samples were collected every three months post-inoculation and immediately prior to euthanasia. To date, eight out of ten inoculated WTD have reached clinical endpoints for CWD and had positive immunoassay results for PrPSc. For the eight deceased WTD, the average incubation period was 24 months (range: 13-33, median 21). Three WTD developed aspiration pneumonia and died suddenly prior to collection of blood. Serum from thirty WTD in the NADC breeding herd were used to establish baseline control levels for NfL. The average serum concentration of NfL in the control population was 6.7 pg/mL (range: 2.1-15.9, median: 5.2). Increases in serum NfL in WTD with CWD was not consistent between deer. In WTD with increased NfL, elevations occurred in the final 25% of the incubation period. The highest endpoint NfL concentration was 395.5 pg/mL in a WTD with a 33.4-month incubation period. In summary, NfL as a biomarker of neurodegeneration in WTD is variable and not all WTD demonstrate large increases in serum; although, some WTD have high endpoint serum NfL levels.

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## **95 Longitudinal detection of prions in specific blood cell populations harvested from white-tailed deer infected with chronic wasting disease.**

Brianne Coleman<sup>1</sup>, Amy Nalls<sup>1</sup>, Erin McNulty<sup>1</sup>, Audrey Sandoval<sup>1</sup>, Caitlyn Kraft<sup>1</sup>, Joseph Westrich<sup>1</sup>, Nathaniel Denkers<sup>1</sup>, Anthony Kincaid<sup>2</sup>, Raymond Bujdoso<sup>3</sup>, Jason Bartz<sup>4</sup>, Candace Mathiason<sup>1</sup>

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### **Abstract**

Chronic wasting disease (CWD) is a terminal, infectious prion disease endemic within captive and free-ranging cervid populations across North America, Korea, and Scandinavia. Big-game hunting and subsequent human consumption of contaminated meat intensifies the need to characterize prion peripheralization in cervids. Prion infectivity has already been detected in blood of prion-infected animals, including deer and humans; yet to be revealed is the temporal profile of specific blood cell subsets associated with prion infection. Using our native white-tailed deer system we have established a reliable, consistent method for isolating blood cell populations throughout disease course from minutes post inoculation to terminal disease, employing density gradient technique for isolation of total peripheral blood mononuclear cells (PBMC); magnetic bead separation for specific blood cell subset extraction, including CD4, CD8, CD14, and B cells; and verification of each cell population by flow cytometry utilizing limited, species-specific antibodies available for cervids. These cell populations were analyzed for the presence of amyloid seeding activity (prions) by real-time quaking-induced conversion (RT-QuIC) assay. To date seeding activity has been identified at or near the time of lymphoid biopsy positivity, with CD14 and B cell populations expressing increasing rates of prion amplification, indications of oscillating T cell interactions over time, and consistent levels of amyloid seeding activity in all populations as disease course enters terminal stage. Our studies are revealing the temporal prion seeding activity in specific blood cell populations across the longitudinal course of CWD infection to better provide an understanding of mechanisms associated with intra-host prion trafficking.

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## **97 From the Dust Bowl to Carbon Sequestration, Landowner Incentives Work. Let's Use Them for Healthy Herds!**

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<sup>1</sup>Wisconsin's Green Fire, Prairie du Sac, WI, USA. <sup>2</sup>Wisconsin's Green Fire, Madison, WI, USA

### **Abstract**

There is a long and successful history of using financial incentives paired with agency technical assistance to encourage landowner cooperation to address pressing conservation challenges. We believe this proven model can be adapted to promote healthy deer herds, and help stem the spread of Chronic Wasting Disease throughout North America.

Since at least the 1930s, financial incentives have been used to conserve soil, retire marginal cropland, restore perennial cover, plant windbreaks, and reforest cutover lands. In recent decades, under the auspices of federal farm bill and other agency programs, the use of incentives has extended to encourage prairie, wetland and woodland restoration, plant buffer strips, cover crops, and pollinator habitat, provide recreational access, and kickoff alternative energy, carbon sequestration and other climate initiatives. Often organized under federal agency authority and funding, these programs are typically adapted to regional conditions and administered at the state and local level.

Field and modeling studies have shown that targeted removal of CWD infected animals provides our best opportunity to manage CWD prevalence and hopefully slow disease spread. Yet maintaining effective targeted removal efforts may quickly overwhelm limited agency staff and resources, particularly if disease is detected beyond the limited perimeter characteristic of early infections.

We propose a locally adaptable and expandable approach to targeted removal that uses financial incentives to enlist the cooperation of landowners, their hunters, and supporting small businesses to help control and manage Chronic Wasting Disease, following the example of nearly a century of successful conservation programs in North America.

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# 98 Isolation of microRNA from deer serum exosomes for potential early detection of chronic wasting disease

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## Abstract

Chronic wasting disease (CWD) is a lethal, degenerative neuronal disease that affects a range of cervid species in the United States. Current diagnostic testing for CWD is highly invasive, often requiring brain or lymph tissue, which are usually recovered post-mortem (brain and nervous system), or via biopsy (lymph tissue). To improve early detection of CWD and provide accurate diagnosis before symptoms are present and shedding of prions occurs, we are developing new methods to screen for CWD biomarkers in cervid blood. Changes in microRNA (miRNA) expression profiles have been associated with other prion diseases such as scrapie and CWD in elk serum. Here we describe a methodology for isolating and extracting miRNA from white-tailed deer serum exosomes, and report initial miRNA profiles for healthy deer. The ability for managers and farmers to surveil wild and domestic cervid populations for CWD using minimally invasive blood sampling has the potential to reduce the reliance on postmortem diagnostics. Effective detection of CWD during early disease could reduce livestock loss from farmed herds and improve the quality of wild deer populations, an important natural resource.

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# 100 Evaluating the diagnostic efficacy of using pooled samples for Chronic Wasting Disease testing and surveillance

Rachel Ruden<sup>1,2</sup>, Monica Hepker<sup>3</sup>, Jianqiang Zhang<sup>2</sup>, Vellareddy Anantharam<sup>3,4</sup>, Anumantha Kanthasamy<sup>3,4</sup>

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## Abstract

Surveillance is the lynchpin of effective response for any natural resource agency tasked with managing Chronic Wasting Disease (CWD) in its wild cervid resource. However, as local prevalence increases and disease management boundaries expand, managers are forced to allocate finite resources between monitoring and intervention. Implementing more efficient testing strategies will help meet not only a growing demand by agencies but a burgeoning demand from the hunting public in North America. Here, we evaluated the efficacy of pooled testing using enzyme-linked immunosorbent assay (ELISA), the current screening test used by veterinary diagnostic laboratories in the United States, and real-time quaking induced conversion (RT-QuIC), an amplification assay that is not yet commercially available. Samples used in this study consisted of medial retropharyngeal lymph nodes routinely collected by the Iowa Department of Natural Resources for the 2019-2020 surveillance effort. Test pools contained tissue from one positive deer diluted in tissue from an increasing number of undetected deer, with each individual contributing an equal tissue volume. ELISA remained positive with pooling thresholds of 1:1, 1:2, 1:4, and 1:9 at a standard volume of tissue homogenate. Moreover, RT-QuIC remained positive with pooling thresholds of 1:1, 1:2, 1:4, 1:9, 1:19, and often 1:49 at a 0.02% tissue dilution. We then used pooled testing with RT-QuIC to re-screen over 3,500 samples from CWD-affected counties and counties that neighbor. Our results suggest that pooled testing can reduce diagnostic costs multifold, and RT-QuIC can be a viable screening test compatible with current field collection standards.

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## 102 Distribution of Chronic Wasting Disease Prions in White-Tailed Deer Hide Following Natural Infection

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### Abstract

Chronic Wasting Disease (CWD) is an infectious disease caused by a misfolded prion protein (PrP<sup>Sc</sup>) that accumulates in all tissues of infected cervids including the skin. As such, much of the focus on tissue distribution of PrP<sup>Sc</sup> has centered on antemortem diagnosis using accessible tissues like the ear. Yet, application to free-ranging cervids is limited. Live capture opportunities tend to be restrictive in scale and scope and would require the dispatch of positive individuals after release. Even as a post-mortem strategy, retropharyngeal lymph nodes will remain the gold standard for early disease detection. However, there are circumstances when an alternative sample type like the hide could be useful, for instance when sampling trophy males, for which damage to the pinna may not be tolerable, or sampling heavily scavenged or decomposed carcasses, for which the pinna may no longer be present. Therefore, understanding the distribution of PrP<sup>Sc</sup> throughout the hide can inform CWD surveillance options. In this study, hide was collected opportunistically from 14 wild white-tailed deer confirmed positive by enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC) of the retropharyngeal lymph node. Hide was then sub-sampled from variable anatomical locations and evaluated by real-time quaking-induced conversion (RT-QuIC) using a protocol previously established for diagnosing Creutzfeldt-Jakob disease in humans. We found notable differences in positivity based on location, sex, and disease status inferred by results from ELISA, IHC, and/or RT-QuIC on the lymph node.

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## 103 Using social media to increase hunter participation in CWD surveillance and response

Anne Justice-Allen

Arizona Game and Fish Department, Phoenix, USA

### Abstract

The Arizona Game and Fish Department has been utilizing a risk-based approach to surveillance for CWD for more than half of the 20 years it has been conducting surveillance. In the eastern high priority region sample collection has been below goals in spite of adequate numbers of animals harvested. In 2020, we began a social media campaign directed at hunters in order to increase hunter participation. To assess the effectiveness of the campaign, we employed market research and advertising companies to develop key messages with the assistance of focus groups. We also assessed hunters pre-campaign knowledge regarding CWD. We ran the campaigns for two consecutive years : 2021 from October 1 to December 31; 2022 from August 9 to September 30. We found that hunters preferred to receive information by email or through the website. The message that resonated best with most hunters was "Keep AZ Herds Healthy. Together we can keep our deer and elk herds healthy and conserve and protect these incredible species." The campaigns consisted of print, email, video, and static and digital display advertising on several platforms (YouTube, Twitter, Facebook). Respondent awareness and knowledge about CWD was significantly increased and the Department was more frequently identified as the source of information after the campaign. Traffic to the Department website was also significantly increased. In the first year, the number of samples collected from eastern Arizona was nearly double of the previous year's submissions but was less than the 5 year average. Unfortunately, we saw a decline in the number of samples in the second year. We attribute the decline to a decrease in the number of permits available and to an inability to have staff in the field during critical times.

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## 105 Factoring Sustainability into the Long-Term Management of Chronic Wasting Disease

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### Abstract

Since its initial detection in 1967, Chronic Wasting Disease (CWD) has established an insidious foothold across much of North America and poses a threat to cervid health globally. With no known cure and a complex epidemiology, CWD can quickly consume state



agencies and provincial governments tasked with managing this disease in its wild cervid resource. As such, it is prudent to prioritize response strategies that can be sustained in perpetuity at the outset. In Iowa, we have been monitoring for CWD since 2002, with our first wild detection in 2013. In 2019, we rolled out a hunter submission pathway to preempt the need for expanded access to testing by our stakeholders. In 2020, we began leveraging our hunting public to achieve targeted removal of deer from endemic disease areas using January incentive seasons. In 2021, we transitioned to weighted surveillance, and despite sampling several thousand fewer deer each year, still detected new positives in counties where the nearest known positive was several counties away (3 counties to date). We also established a 3-5 year phase out plan for enhanced surveillance around both wild and captive positives, recognizing that not every spark will result in disease establishment (3 areas have been phased out). Finally, we are taking a unique approach to public education by catalyzing local change agents through our CWD Ambassador program. By focusing on CWD management strategies that are scalable and adaptive to uncertain risk landscapes, we can tailor responses to our agency's needs that slow the spread sustainably.

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## 109 Farmed Cervid Unmanned Aircraft System (UAS) Surveillance

DREW CRISLER, JOHN NELSON, ELSIE MCCOY, NATHAN BROWN

KANSAS DEPARTMENT OF AGRICULTURE, MANHATTAN, USA

### Abstract

Herd counts are challenging for cervid producers; especially those that utilize large, native range acreages rather than pens or lots to manage their herd. Despite being classified as domesticated, these cervids display behavior similar to wild cervids, always maintaining distance between themselves and any perceived threats (i.e., producers, veterinarians, regulatory staff).

Another challenge for our agency is our responsibility to declare to the Kansas Department of Wildlife, Parks, and Tourism that all wild cervids have been cleared from a newly fenced farmed cervid facility. Cervids instinctually tend to hide from humans and blend in well with their natural surroundings making it challenging to identify and remove cervids prior to final completion of the perimeter fence.

The UAS, equipped with both a high-definition color camera and a thermal imaging camera, is utilized for the project. Despite large acreage, dense foliage, or rugged terrain that may be inaccessible on foot, this system allows us to survey and access an entire facility in a short period of time. Data collected assists in determining herd inventory and provides an indication of current herd health. In the case of new facilities, use of the system ensures no wild cervids are located within the boundaries of the facility prior to populating with farmed cervids.

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## 111 SAGOTR, a Strain and Genetics Online Tissue Repository for Chronic Wasting Disease

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### Abstract

The ability to build our knowledge on infectious disease regarding genetics, biochemistry, host ranges, and disease properties is contingent on available research materials. Chronic wasting disease (CWD) is a prion disease that affects Cervidae species. Since CWD was first identified in the 1960s in the United States, it has been documented in 30 US states and three Canadian provinces in both captive and wild cervids. Different protein conformations of infectious prions result in various disease phenotypes which are used to characterize different prion strains. However, the knowledge about CWD strains is still fragmented, and one of the main obstacles is lack of a centralized collection of CWD-infected tissues among state agencies and research institutes. To attempt to overcome this impediment, we established the Strain and Genetics Online Tissue Repository (SAGOTR) for CWD. This virtual repository is freely available to researchers to upload sample metadata or to access tissue from cooperating organizations collected on a large geographic scale in North America. Important metadata (coordinates for collection location, species, tissue type, reference, and testing results) will be linked to each tissue sample. SAGOTR provides maps of locations of standardized CWD-infected and uninfected tissue resources and polymorphism of the host prion protein gene for each tissue available. Monitoring these data over time will provide important information regarding CWD strain types, their dynamics, distribution, and will allow for the identification of novel emerging strains that may have altered pathogenicity and/or zoonotic potential compared to currently circulating CWD strains.

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## 112 Why it is so hard? The sociological and political challenges to minimizing the impacts of CWD on deer populations

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<sup>1</sup>Missouri Department of Conservation, Jefferson City, USA. <sup>2</sup>Ohio DNR Division of Wildlife, Athens, USA

### Abstract

Reducing the spread of chronic wasting disease (CWD) and minimizing the impacts on deer populations is conceptually simple. Disrupt disease transmission by eliminating infected animals at a rate higher than it can be spread and eliminate the activities that result in new introductions of the disease. However, the social and political realities make the management of CWD one of the greatest challenges facing the conservation community. We explore the most significant barriers limiting state fish and wildlife agency's ability to successfully implement meaningful management actions to address the continued spread of CWD in free-ranging cervids. Lack of funding and inconsistent messaging among states are often cited as major contributors to the continued spread of this disease. We suggest that it is vastly more complicated, as it involves agency priorities and the reality that managing CWD often pits agencies squarely against several key tenets of the North American Model. Slowing the spread of CWD will not come until agencies can build support and move forward with regularly make what have traditionally been unpopular decisions. Unfortunately, people have paid dearly to build this resource, and in the absence of paradigm shifting breakthroughs, agencies need to largely dismantle it to even keep up with CWD. We need to reduce populations and shoot most bucks before they reach 2.5 years of age. While the science is clear about the deer that represent the greatest risk, we continue to promote and protect them. Finally, we are losing hunters nearly as fast as our deer herd is growing. Unless we are able to adjust the social and political will, we are simply putting new dots on an old map and agencies will continue to struggle to find a way to ensure the future of deer populations in North America.

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## 113 Indications of population impacts and declining hunter participation in the South Saskatchewan River Valley: A growing management concern

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<sup>3</sup>University of Saskatchewan/CWHC, Saskatoon, Canada

### Abstract

Chronic wasting disease (CWD) continues to spread geographically and increase in prevalence in Saskatchewan mule deer populations. In 2021-22, the province-wide prevalence rate of CWD in male mule deer was 48% ( $\alpha = 0.05$ ,  $n = 654$ , +/- 4%). The prevalence rate of CWD in female mule deer was 24% ( $\alpha = 0.05$ ,  $n = 600$ , +/- 3%). The highest prevalence rates of CWD in the province remains in the South Saskatchewan River valley (SSRV) region, where male mule deer prevalence estimates have been observed as high as 79% ( $\alpha = 0.05$ ,  $n = 67$ , +/- 10) and 52% in females ( $\alpha = 0.05$ ,  $n = 31$ , +/- 17). Mule deer population survey data in the SSRV region suggests that mule deer populations are declining and there are fewer mature animals, presumably due to elevated mortality associated with CWD infection. We have found the number of either-sex and antlerless mule deer limited entry hunting applications, an index for hunter interest, has declined over time, presumably due to high prevalence rates in the SSRV region. This finding adds to the growing body of work demonstrating that CWD is an important factor that can influence hunter behaviour. Extremely high prevalence of CWD paired with declining mule deer populations and hunter interest has created an unprecedented challenge to mule deer management in the SSRV of Saskatchewan.

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## 114 Chronic Wasting Disease Binding to Agricultural Crops

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### Abstract

Chronic wasting disease (CWD) prions are shed from infected cervids via body secretions and excretions. The CWD prions bind to vegetation and soil, potentially contributing to prion accumulation and persistence in the environment. In this study, we evaluated the interactions of CWD prions with common crops including peas, canola, and wheat. We also identified pesticides that disrupted such interactions. When leaves of various plant species are contaminated with CWD prions for 24 hours and then washed with water, the majority of the PrP<sup>CWD</sup>, as detected by western blot, is associated with the leaf (i.e., the bound fraction). Little to no PrP<sup>CWD</sup> was in the unbound (wash) fractions. We also examined the ability of fungicides and insecticides to impact CWD prion binding and adherence.

Pre-treatment of vegetation resulted in less binding to the leaf surfaces, while PrP<sup>CWD</sup> was increased in the unbound fractions with fungicidal compounds. Similarly, treatment of vegetation with urea post-application of the PrP<sup>CWD</sup> prions to the leaf surfaces showed similar results. Our data suggest that although crops can bind CWD prions, serving as a reservoir for CWD; binding can be mitigated by treatment with specific fungicides.

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## 115 CWD prions in the interdigital glands of mule deer, white-tailed deer and moose

Anthony Ness<sup>1</sup>, [Aradhana Jacob](#)<sup>1</sup>, Diana Martinez Moreno<sup>1</sup>, Doris Zeng<sup>2</sup>, Alsu Kuznetsova<sup>1</sup>, Alicia Otero García<sup>3</sup>, Chiye Kim<sup>1</sup>, Kelsey Saboraki<sup>4</sup>, Susan Lingle<sup>4</sup>, Margo Pybus<sup>5</sup>, Judd Aiken<sup>1</sup>, Sabine Gilch<sup>2</sup>, Debbie McKenzie<sup>1</sup>

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<sup>4</sup>University of Winnipeg, Winnipeg, Canada. <sup>5</sup>Government of Alberta, Edmonton, Canada

### Abstract

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy that fatally affects cervids. CWD is transmitted directly (infected animal-animal) and indirectly (pathogen shedding into the environment via body secretions and decomposing carcasses). Although shedding via saliva, urine and feces is well-documented, we hypothesize the secretions from other glands may also contribute to the contamination of the environment.

We characterized the presence and distribution of PrPC in six integumentary (forehead, preorbital, lateral vestibular nasal, tarsal, metatarsal, and hind interdigital glands) and two non-integumentary tissues (parotid gland and vomeronasal organ) of hunter-harvested mule deer and white-tailed deer. The presence of PrPC in these glands supports the idea that PrPCWD could accumulate in these tissues. Further investigation of the interdigital glands, the sac-like exocrine structure located between the digits of the hooves, revealed the presence of PrPCWD within the gland. A blinded analysis of deer interdigital glands by real-time quake-induced conversion (RT-QuIC) accurately identified CWD-infected animals. Analysis of additional samples from white-tailed deer, moose and mule deer interdigital glands and comparison to CWD test results will provide a more complete understanding of the role interdigital glands may play in indirect transmission of prions via the environment.

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## 116 A prion in a poop-stack: detection tools and carnivore-based surveillance of chronic wasting disease

[Heather N'te Inzalaco](#)<sup>1</sup>, Ellen E. Brandell<sup>1</sup>, Sam Wilson<sup>2</sup>, Matt Hunsaker<sup>1</sup>, Dan R. Stahler<sup>3</sup>, Kaylee Woelfel<sup>4</sup>, Dan P. Walsh<sup>5</sup>, Todd Nordeen<sup>2</sup>, Dan J. Storm<sup>6</sup>, Stuart S. Lichtenberg<sup>7</sup>, Wendy C. Turner<sup>8</sup>

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### Abstract

Chronic wasting disease (CWD) is a highly contagious, fatal neurodegenerative disease caused by infectious prions (PrPCWD) affecting wild and captive cervids. Although recent experimental feeding studies have demonstrated infectious prions in feces of crows (*Corvus brachyrhynchos*), coyotes (*Canis latrans*), and cougar (*Puma concolor*), the role of scavengers and predators in CWD epidemiology remains poorly understood. Here we adapted tools that can help more accurately capture how cervid consumers may be involved in CWD epidemiology to advance surveillance approaches, disease modeling, and management of CWD. Using the real-time quaking-induced conversion (RT-QuIC) assay to test our extraction process, we demonstrate detection of PrPCWD from experimental spiking of PrPCWD into predator and scavenger feces sourced from CWD-free populations and captive facilities. Application of RT-QuIC to detect PrPCWD from feces of free-ranging predators and scavengers revealed that i) PrPCWD is detectable from scats of free-ranging carnivores using RT-QuIC and ii) CWD prevalence rates in carnivore scats reflect rates observed in the corresponding cervid population. Our findings provide an important preliminary glimpse into the implications of a community-level factor that may have a cascading influence on structuring both broad and endemic CWD prevalence.

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# 117 Artificial mineral sites that pre-date endemic chronic wasting disease become prion hotspots

Heather N'te Inzalaco<sup>1,1</sup>, Marie L. Gilbertson<sup>1</sup>, Stephanie J. Katircioglu<sup>1</sup>, Kenny Lepard<sup>2</sup>, Jordan McEarl<sup>2</sup>, Austin Bibb<sup>2</sup>, Jeremy Dennison<sup>2</sup>, Dan Grove<sup>3</sup>, Allen Houston<sup>4</sup>, Dan J. Storm<sup>5</sup>, Dan P. Walsh<sup>6</sup>, Wendy C. Turner<sup>7</sup>, Stuart S. Lichtenberg<sup>8</sup>

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## Abstract

Chronic wasting disease (CWD) is a highly contagious, fatal neurodegenerative disease caused by infectious prions (PrPCWD) affecting wild and captive cervids. As CWD continues to spread broadly over North America, factors influencing geographic expansion remain poorly understood. CWD-infected cervids shed infectious prions in urine, feces, and saliva. Activities that result in cervids aggregating and shedding PrPCWD may result in 'hot spots' of environmental PrPCWD deposition, however empirical data regarding the impact of deer attractants on environmental PrPCWD deposition is lacking. Filling this knowledge gap could inform deer and disease management. In 2018, the southwest region of Tennessee, U.S.A. experienced a CWD outbreak. The Ames Research and Educational Center property, centrally located within the CWD zone of southwest Tennessee, contains 49 historical mineral supplementation sites that were decommissioned in 2012. Here, we demonstrate that 32 of the 49 (65%) mineral sites within Ames established prior to the regional CWD outbreak, serve as foci of environmental PrPCWD contamination. Detection of PrPCWD in soils from these artificial mineral sites was dependent on site-specific management efforts. Soil physical properties were very similar across sites and no correlation between PrPCWD detection and soil physical properties was found. The detection of PrPCWD in soils at attractant sites within an endemic CWD zone significantly advances our understanding of environmental PrPCWD accumulation dynamics, providing valuable information for advancing adaptive CWD management approaches.

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# 118 Potential Application of Detection Dogs in Chronic Wasting Disease Management

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<sup>1</sup>Penn Vet Wildlife Futures Program, Kennett Square, USA. <sup>2</sup>Pennsylvania Game Commission, Harrisburg, USA. <sup>3</sup>Penn Vet Working Dog Center, Philadelphia, USA

## Abstract

Chronic wasting disease (CWD) has changed the landscape of hunting in Pennsylvania. A threat to deer and elk, this contagious, always fatal disease was detected in Pennsylvania for the first time in 2012. The long incubation period of CWD makes it difficult to detect, which is why testing and surveillance are so important. There may be a new tool on the horizon to help with that.

Supported by a pilot study from the Penn Vet Working Dog Center that showed trained detection canines could be used as an antemortem test for CWD, the University of Pennsylvania's Wildlife Futures Program (WFP) and the Pennsylvania Game Commission (PGC) are working with additional support from the Pennsylvania Department of Agriculture (PDA) to develop a detection dog program capable of detecting CWD on the landscape. The goal of this program is to enhance and assist with PGC CWD response and management activities.

Potential management applications of this program include use in Containment Zones, use in areas of changed DMA status, use in the case of clinical suspects in a new area (in or on a boundary), in areas of lower-than-expected disease prevalence, and to surveil the status of collared deer based on their tracked locations.

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# 120 Delayed Wisc-1 CWD lymphotropic replication may explain differences in neuroinvasion between host expressing G96 or S96 cellular prion protein polymorphisms.

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## Abstract

The main host factor affecting the likelihood of cervids of becoming infected and developing chronic wasting disease (CWD) is the primary structure of the cellular prion protein (PrP<sup>C</sup>). Single amino acid polymorphism in PrP<sup>C</sup> can impair continuous generation of PrP<sup>CWD</sup>. For example, the serine polymorphism at amino acid 96 (S96) of PrP<sup>C</sup> slows progression of CWD in white-tailed deer. In tg60 mice, S96-PrP<sup>C</sup> protects against prion disease following oral, intraperitoneal and intracranial exposure with the Wisc-1 CWD strain. Interestingly, the H95<sup>+</sup> strain, characterized by a defined PrP<sup>CWD</sup> conformational spectra, PrP<sup>res</sup> glycotype and stable disease neuropathology by the intracranial route, failed to produce full attack rate and phenotypically similar prion disease by the oral and intraperitoneal routes. *In vitro* PrP<sup>CWD</sup> amplification by PMCA shows H95<sup>+</sup> can be perpetually propagated with S96-PrP<sup>C</sup> substrate and indicates Wisc-1 loses replicative capacity once encoded on S96-PrP<sup>CWD</sup>. Together, this data suggests that impaired neuroinvasion in hosts expressing S96-PrP<sup>C</sup> slows disease progression.

To evaluate the impact of PrP<sup>C</sup> polymorphisms and route of exposure on strain-tissue tropism, PrP<sup>res</sup> glycotype and disease progression, we inoculated cohorts of tg33 and tg60 mice with Wisc-1 or H95<sup>+</sup>, by the intracerebral, intraperitoneal and oral routes. To compare the rate of lymphotropic accumulation in spleen, mesenteric lymph nodes and Peyer patches, samples were taken at specific time points for PrP<sup>res</sup> evaluation. Western blot analysis demonstrates Wisc-1 PrP<sup>CWD</sup> accumulation in the spleen of intraperitoneally inoculated tg33 mice (i.e., homologous transmission) as early as 1-month post-exposure. Comparable levels of PrP<sup>res</sup> in tg60 mice spleens were detected by 6 months post exposure. These data suggest that Wisc-1 lymphoid replication is impaired in hosts expressing S96-PrP<sup>C</sup> compared to hosts expressing G96-PrP<sup>C</sup>.

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# 121 Scavenger community dynamics at Cervidae carcasses in an endemic chronic wasting disease area

Kelly Russo<sup>1</sup>, George Wittemyer<sup>1</sup>, Kurt Vercauteren<sup>2</sup>

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## Abstract

Wildlife species harbor and transmit pathogens and parasites throughout their lives. Scavenger species are exposed to these pathogens and parasites when visiting and consuming the carcasses of infected individuals. Despite the transmission potential inherent at these locations, critical gaps remain in our understanding of the role scavenging species play in disease dynamics, particularly in the context of chronic wasting disease (CWD). Pathogenic prion proteins (PrP<sup>Sc</sup>), the agent driving CWD infections in members of the Cervidae family, have prolonged environmental latency, potentially persisting in the environment for more than a decade. Prions remain infectious after passage through the digestive tracts of scavenging birds and mammals, however the degree to which scavengers sequester or spread prions and thus CWD remains an open question. As CWD continues to emerge in new locations throughout North America, it is imperative to understand all mechanisms of spread and maintenance in the environment. To investigate scavenging community dynamics at Cervidae carcasses in northern Colorado, we monitored carcasses of vehicle-killed deer and elk, as well as gut piles from hunter-harvested deer and elk, with camera traps beginning in January 2022. From images captured, we calculated species richness, relative abundance, temporal activity patterns, and distinguished carcass visitors from carcass consumers. Study results will inform our understanding of the role that scavenger species play in CWD transmission, and provide insight into Cervidae carcass utilization, patterns of scavenger consumption, and interspecific interactions of scavengers.

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## 122 Detection of Chronic Wasting Disease in Environmental Matrices at a White-Tailed Deer Carcass Dumpsite

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### Abstract

Misfolded, infectious prion proteins, PrPTSE, are able to effectively bind to the constituents of environmental matrices such as soil and remain infectious. Since environmental samples have varying physical and chemical properties, testing these samples using real-time quaking induced conversion (RT-QuIC) assay, an ultra-sensitive prion amplification assay, requires characterization of environmental background seeding activity. Determining seeding activity of environmental matrices and how they differ from PrPTSE contaminated samples allows the binomial classification of samples as prion detections versus non-detections. In this study, we worked to optimize RT-QuIC for the detection of prions in soils from a carcass dumpsite associated with a CWD-positive white-tailed deer (*Odocoileus virginianus*, WTD) facility in Minnesota. To determine background RT-QuIC seeding activity for the soils found in the area, we collected soil samples (orders Histosol and Alfisol) 7 cm deep every meter along four 10-20 meter transects outside of the dumpsite where prions were not expected to be found. We then spiked the samples with CWD-positive WTD lymph node tissue to establish CWD-detected distributions for our site's soil orders. We will use the RT-QuIC seeding distributions of these sample sets (background versus spiked) to establish a threshold for binomial classification of detections versus non-detections. Moving forward, soil samples collected from the dumpsite will be tested and classified by the established thresholds to characterize prion contamination of the site. Altogether, these investigations allow us to evaluate RT-QuIC performance for detecting CWD prions in complex environmental matrices.

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## 123 Building a CWD surveillance information system for New York State

Nicholas Hollingshead<sup>1</sup>, Paul Adams<sup>2</sup>, Cara Them<sup>1</sup>, Brenda Hanley<sup>1</sup>, Rachel Abbott<sup>1</sup>, Md Sohel Ahmed<sup>1</sup>, Krysten Schuler<sup>1</sup>

<sup>1</sup>Cornell University, Ithaca, USA. <sup>2</sup>DJ Case & Associates, Mishawaka, USA

### Abstract

Chronic wasting disease (CWD) continues to spread throughout North America despite significant resources committed by wildlife agencies to CWD surveillance, outbreak response, and management. For many wildlife agencies, the strain on personnel and financial costs are substantial. Therefore, it is critical that activities related to CWD are conducted as efficiently and effectively as possible. In 2020, the New York State Department of Environmental Conservation (NYSDEC) joined the Surveillance Optimization Project for Chronic Wasting Disease (SOP4CWD), a multi-institution collaboration that uses mathematical modeling and data science to address the challenges of CWD surveillance and response. The project is led by the Cornell Wildlife Health Lab at Cornell University and the Boone and Crockett Quantitative Wildlife Center at Michigan State University. In Fall 2022, SOP4CWD created the CWD Data Warehouse, a shared online platform that integrates CWD data management, modeling, analysis, and visualization that is available to all wildlife agencies for free. NYSDEC adopted the Warehouse as its primary CWD surveillance data management hub. Combined with ESRI ArcGIS web and mobile applications, and cloud computing services, NYSDEC has created a cohesive CWD data management system that has improved data quality, eliminated inefficiencies, and increased situational awareness. Further, the new system would also allow NYSDEC to ramp up its sampling effort in the case of a CWD outbreak. In this presentation, we will describe the system and explain how the technologies and solutions developed for NYSDEC may be readily adopted by other state wildlife agencies for CWD surveillance and other wildlife management applications.

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## 124 Herd-level risk factors associated with chronic wasting disease-positive herd status in Minnesota, Pennsylvania, and Wisconsin cervid farms

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### Abstract

Little research has been performed to identify Chronic Wasting Disease (CWD) transmission pathways to farmed cervid operations other than through farmed cervid movements. Identification of risk factors associated with CWD-positive cervid farm status would facilitate better understanding of transmission and is critical to inform improved disease prevention and surveillance. The objective of

this USDA-APHIS funded project (2020-2022) was to evaluate biosecurity risks related to introduction of CWD to farmed cervid operations in Minnesota, Pennsylvania, and Wisconsin. We used a matched case-control epidemiological observational study where herd status was identified through participation in mandatory State CWD herd program. Data was collected from voluntarily participating white-tailed deer herds using a questionnaire and state animal health agency databases. Associations between CWD herd status and each variable were identified using univariable analyses (double-sided t-test or Cochran-Mantel-Haenszel test). Variables from univariable analyses that represented different biological transmission pathways were evaluated using logistic regression. Univariable analyses identified associations between CWD-positive herd status and variables representing different transmission pathways, including direct contact with infected farmed cervids (introduction of cervid from a farm later found infected) and several variables representing indirect contact with infected wild cervids. Results identified indicate the importance of transmission to cervid operations through different pathways, including indirect contact pathways with infected wild cervids. Further studies are needed to confirm and clarify understanding of indirect pathways to allow development of improved biosecurity practices to prevent CWD introduction to cervid operations.

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## **126 Informing response to chronic wasting disease (CWD) in Wisconsin's wild cervids using structured decision making and systems modeling**

Jennifer Price Tack<sup>1</sup>, Christine Anhalt-Depies<sup>2</sup>, Thomas Fiddaman<sup>3</sup>, Curtis Rollman<sup>4</sup>, Bryan Richards<sup>5</sup>, Daniel Storm<sup>6</sup>, Daniel Walsh<sup>5,7</sup>, C. LeAnn White<sup>5</sup>

<sup>1</sup>Wisconsin Department of Natural Resources, Office of Applied Science, Rhinelander, USA. <sup>2</sup>Wisconsin Department of Natural Resources, Office of Applied Science, Madison, USA. <sup>3</sup>Ventana Systems, Inc, Harvard, USA. <sup>4</sup>Wisconsin Department of Natural Resources, Rhinelander, USA. <sup>5</sup>USGS National Wildlife Health Center, Madison, USA. <sup>6</sup>Wisconsin Department of Natural Resources, Office of Applied Science, Eau Claire, USA. <sup>7</sup>USGS Montana Cooperative Wildlife Research Unit, Missoula, USA

### **Abstract**

The Wisconsin Department of Natural Resources (DNR) recently reviewed its Chronic Wasting Disease (CWD) Response Plan to 1) determine whether the plan is achieving its goal, and 2) revise the plan as needed. To conduct the review, the DNR used structured decision making and a systems approach led by DNR decision analysts, researchers at the USGS National Wildlife Health Center, USGS Montana Cooperative Wildlife Research Unit, and modeling consultants, Ventana Systems, Inc. The agency established a Response Plan Review Committee with representatives from the agency, conservation and hunting organizations, tribes, and industries impacted by CWD and CWD response. The Committee identified their values related to CWD and a suite of actions for the DNR to consider, i.e., baiting and feeding bans, changes to harvest regulations, targeted removals. The Committee then developed a set of alternative response plans, each comprised of a unique set of actions. Using the systems model, developed through participatory modeling, the Committee evaluated how the alternative plans would affect CWD and other stakeholder objectives. The results were summarized into a consequences table that explicitly highlights tradeoffs among objectives. For example, achieving desired reductions in CWD prevalence rates required implementing socially unpopular and expensive actions, including targeted removals. The unique pairing of structured decision making and a systems approach to inform the Review provides a powerful framework to inform agency action and identify leverage points that may exist outside of the agency's authority to implement for improving deer health while explicitly involving stakeholders and accounting for socio-political challenges.

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## **128 Prion detection in the blood of free-ranging CWD-infected white-tailed deer**

Amy Nalls<sup>1</sup>, Erin McNulty<sup>1</sup>, Nathaniel Denkers<sup>1</sup>, Devon Trujillo<sup>1</sup>, Samantha Scherner<sup>1</sup>, Ethan Barton<sup>2</sup>, Christopher Cleveland<sup>3</sup>, Natalie Stilwell<sup>3</sup>, James Crum<sup>4</sup>, Daniel Grove<sup>5</sup>, Jeremy Dennison<sup>6</sup>, Jennifer Ballard<sup>7</sup>, Edward Hoover<sup>1</sup>, Mark Ruder<sup>3</sup>, Candace Mathiason<sup>1</sup>

<sup>1</sup>Colorado State University, Fort Collins, USA. <sup>2</sup>West Virginia Division of Natural Resources, Romney, USA. <sup>3</sup>University of Georgia, Athens, USA. <sup>4</sup>West Virginia Division of Natural Resources, Elkin, USA. <sup>5</sup>University of Tennessee Extension, Nashville, USA. <sup>6</sup>Tennessee Wildlife Resources Agency, Jackson, USA. <sup>7</sup>Arkansas Game and Fish Commission, Little Rock, USA

### **Abstract**

Infectious prions circulate in the blood of animals with prion disease, including cervids (chronic wasting disease), sheep (scrapie), cattle (bovine spongiform encephalopathy), and humans (Creutzfeldt-Jakob disease). The development of rapid, sensitive, and specific assays to detect blood-borne prions has been challenging due to presumed low concentrations of hematogenous prions and/or inhibitors present in blood. Our laboratory has performed extensive chronic wasting disease (CWD) studies in the natural white-tailed deer host, providing a unique repository of serially collected samples throughout the disease course. This repository, in addition to ongoing CWD studies in deer, has permitted us the opportunity to optimize blood cell isolation techniques and prion detection assays. We have demonstrated the temporal presence of prions in blood buffy coat cells isolated from CWD-inoculated white-tailed deer, efficiently detecting prions in the blood of animals during both subclinical and clinical stages of disease. Through a collaboration with Southeastern Cooperative Wildlife Disease Study, we received field-collected anticoagulated blood samples from n=34 free-ranging white-tailed deer collected in CWD endemic areas of Arkansas, Tennessee and West Virginia. 1ml of anticoagulated blood contained

sufficient buffy coat cells for our in vitro detection assay. We revealed prion positivity in 27% of deer blood samples collected in Arkansas, 78% in Tennessee, and 44% in West Virginia, and saw a strong correlation with tissue positivity. Future work will explore the potential of this assay in diagnostics and surveillance of additional free-ranging cervid populations.

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## 129 Investigating potential inhibitory roles of organic copper and zinc in CWD prion protein misfolding and propagation

Joseph Hediger<sup>1</sup>, Peter Larsen<sup>2</sup>, Jason Bartz<sup>3</sup>, Randy DeYoung<sup>1</sup>, David Hewitt<sup>1</sup>, Mitch Lockwood<sup>4</sup>, Alynn Martin<sup>1</sup>, J. Hunter Reed<sup>4</sup>, Marc Schwabenlander<sup>2</sup>, Tiffany Wolf<sup>5</sup>, Michael Cherry<sup>1</sup>

<sup>1</sup>Caesar Kleberg Wildlife Research Institute, Kingsville, TX, USA. <sup>2</sup>Minnesota Center for Prion Research and Outreach, St. Paul, MN, USA. <sup>3</sup>Creighton University, Omaha, NE, USA. <sup>4</sup>Texas Parks and Wildlife Department, Austin, TX, USA. <sup>5</sup>University of Minnesota, St. Paul, MN, USA

### Abstract

Positive cases of Chronic Wasting Disease (CWD) are spreading rapidly across the United States, Canada, and Europe. Although a treatment is currently unavailable for CWD, there is potential for molecules to bind to misfolded prions preventing or slowing subsequent protein misfolding. In a rodent model, orally administered copper inhibited the misfolding and propagation of infectious proteins. However, it is yet unknown how mineral status influences disease progression in cervids. We are working to determine if copper (Cu-AA) and zinc (Zn-AA) concentrations in target organs influence the propagation of misfolded CWD prions (PrP<sup>CWD</sup>). To do this we harvested 150 mature white-tailed deer (*Odocoileus virginianus*) spanning a gradient of Cu-AA and Zn-AA bioavailability in Texas, USA. Samples, including liver, brain, distal ileum, medial retropharyngeal lymph nodes, and tongue, were collected from 1) deer in South Texas with natural nutrition, 2) free-ranging deer with access to supplemental feed enhanced with Cu-AA and Zn-AA, and 3) captive deer fed exclusively feed with Cu-AA and Zn-AA supplementation. We will use prion misfolding cyclic amplification to evaluate the role of Cu-AA and Zn-AA concentrations in brain and lymphoid tissue in reducing PrP<sup>CWD</sup>, while accounting for CWD status and genetic susceptibility to CWD. This research has the potential to identify molecules that may serve as novel management options for CWD treatment and prevention.

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## 130 Evaluating the interaction of emerging diseases on white-tailed deer populations

Miranda Strasburg<sup>1</sup>, Sonja Christensen<sup>2</sup>

<sup>1</sup>Michigan State University, East Lansing, USA. <sup>2</sup>East Lansing, East Lansing, USA

### Abstract

Disease co-occurrence in wildlife populations is common yet understudied. In the case of disease-caused mortality, the mortality attributed to one disease has the potential to buffer populations against subsequent alternative disease outbreaks by reducing populations and thus, contacts needed to sustain disease transmission. However, substantial disease-driven population declines may also prevent populations from recovering, leading to localized extinctions. Hemorrhagic disease (HD) is a vector-transmitted, viral disease in white-tailed deer (WTD) and, similarly to CWD, has increased in frequency and distribution in the USA. However, unlike CWD, which progresses slowly, HD can cause mortality only days after infection. Hemorrhagic disease outbreaks can result in substantial localized mortality events in WTD near vector habitats such as wetlands and may reduce local deer densities and consequent CWD transmission. The objective of our study was to evaluate the potential for HD outbreaks to buffer CWD risk where the disease might co-occur. The occurrence of both CWD and HD across counties in the upper Midwest has increased over the last 2 decades with high potential for disease overlap in Wisconsin, Illinois, and Michigan. Using an agent-based modeling approach, we found that frequent, intense HD outbreaks have the potential to mitigate CWD risk, especially if those outbreaks occur shortly after CWD introduction. However, HD outbreaks that do not result in substantial WTD mortality are unlikely to impact CWD or WTD population dynamics. Severe HD outbreaks may reduce CWD cases and could present an opportunity for managers to boost CWD control initiatives in a post-HD outbreak year.

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# 131 Novel strain properties of emergent prions causing chronic wasting disease in red deer from Norway

Xutong Shi<sup>1,2</sup>, Julianna Sun<sup>1</sup>, Sehun Kim<sup>1</sup>, Jenna Crowell<sup>1</sup>, Bailey Webster<sup>1</sup>, Glenn Telling<sup>1,2</sup>

<sup>1</sup>Prion Research Center (PRC), the Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, USA. <sup>2</sup>Program in Cell and Molecular Biology, Colorado State University, Fort Collins, USA

## Abstract

Since 2016, CWD has been diagnosed in free-ranging reindeer, moose and red deer in Norway, Finland and Sweden. Our previous studies have shown that prion strains causing CWD in Norway moose, Norway reindeer, and Finland moose are different from each other and from prions causing CWD in cervids from North America. We have also showed that so-called gene-targeted (Gt) mice expressing cervid prion protein (PrP) under the control of mouse PrP gene transcriptional elements are susceptible to these distinct Nordic and North American CWD prions and that their propagation is influenced by naturally-occurring amino acid differences at residue 226 of PrP. Here we investigated the transmission properties of CWD prions causing CWD in Norway red deer in Gt mice. The properties of these emergent prions as assessed by susceptibility in Gt mice of different cervid PrP genotypes, western blotting, neuropathology and using various in vitro amplification platforms are distinct yet again to those causing disease in other Nordic or North American cervids. The in vivo transmission of these red deer CWD prions is facilitated by expression of glutamate at residue 226, the predominant allele expressed in elk species. However, transmission and in vitro amplification of these prions is relatively inefficient compared to other Nordic or North American CWD strains, with predominantly subclinical disease occurring in the presence of high levels of prion accumulation in the brains of Gt mice after prolonged incubation times. Our findings are consistent with a growing portfolio of emergent CWD strains in Nordic countries which contrasts the relatively consistent properties of CWD in North American cervids.

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# 132 Exploring the utility of RT-QuIC for detection of CWD in naturally infected, free-ranging white-tailed deer in CWD-endemic areas of the Southeastern United States

Ethan Barton<sup>1</sup>, Amy Nalls<sup>2</sup>, Erin McNulty<sup>2</sup>, Audrey Sandoval<sup>2</sup>, Devon Trujillo<sup>2</sup>, Nathaniel Denkers<sup>2</sup>, Samantha Scherner<sup>2</sup>, Christopher Cleveland<sup>3</sup>, Natalie Stilwell<sup>3</sup>, James Crum<sup>1</sup>, Daniel Grove<sup>4</sup>, Jeremy Dennison<sup>5</sup>, Jennifer Ballard<sup>6</sup>, Edward Hoover<sup>2</sup>, Candace Mathiason<sup>2</sup>, Mark Ruder<sup>3</sup>

<sup>1</sup>West Virginia Division of Natural Resources, Elkins, USA. <sup>2</sup>Colorado State University, Fort Collins, USA. <sup>3</sup>Southeastern Cooperative Wildlife Disease Study, Athens, USA. <sup>4</sup>University of Tennessee Extension, Nashville, USA. <sup>5</sup>Tennessee Wildlife Resources Agency, Jackson, USA. <sup>6</sup>Arkansas Game and Fish Commission, Little Rock, USA

## Abstract

Amplification assays (e.g., real-time quaking induced conversion (RT-QuIC)) demonstrate promise for improved detection of CWD in free-ranging cervids. Wildlife managers and researchers rarely have opportunities for serial sampling and typically rely upon single timepoint sampling during capture events or at harvest. During 2020 and 2021, we collected retropharyngeal lymph nodes (RPLN), tonsils, obex, rectoanal mucosa-associated lymphoid tissue (RAMALT), blood, urine, and saliva from 37 free-ranging white-tailed deer within endemic areas of Arkansas (n=11), Tennessee (n=16), and West Virginia (n=10). To better understand utility and limitations of such sample types, we tested tissues by immunohistochemistry (IHC) and RT-QuIC and fluids by RT-QuIC. Overall, IHC on RPLN identified 17 CWD-positive animals; RT-QuIC failed to detect one of these but detected prion in six additional deer. In tonsil, RT-QuIC detected eight animals not identified by IHC. We also explored detection of CWD in antemortem sample types (e.g., RAMALT, blood) relative to detection in RPLN. Combined RT-QuIC testing of buffy coat cells and RAMALT successfully identified 94.1% of RPLN IHC-positive deer. However, RT-QuIC on RAMALT alone identified 76.5% and IHC on RAMALT identified only 50%, suggesting the potential utility of multiple antemortem sample types to increase likelihood of detection. Finally, we explored shedding profiles in urine and saliva through RT-QuIC testing. Prion was detected in saliva from four deer that tested positive (by RT-QuIC and IHC) in multiple tissues but only one deer had evidence of prion in urine. Such variable shedding profiles are consistent with those observed in experimental studies.

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## **134 Emergent prion disease in Swedish moose causes remarkably rapid disease in mice.**

Diana Lowe, Julianna Sun, Sehun Kim, Jenna Crowell, Emma Raisley, Bailey Webster, Glenn Telling

Colorado State University, Fort Collins, USA

### **Abstract**

Chronic Wasting Disease (CWD) is a neurodegenerative disease of cervids (deer, elk, moose, reindeer and red deer) caused by PrP<sup>Sc</sup> an aberrantly folded form of the normal, host-encoded prion protein (PrP). CWD has a long incubation period and is extremely contagious, constituting a growing endemic situation for cervids in North America. The zoonotic risk of CWD is uncertain, but animal prion diseases have shown a potential to cross the species barrier, illustrated by the outbreak of mad cow disease in the United Kingdom in the mid-1980s. In addition, CWD has recently emerged in Northern Europe and recent studies from our research group demonstrate, that it constitutes a sporadic, unstable type of CWD, with distinct properties from its North American counterpart, posing an unpredictable threat to wildlife in the region and potentially to humans. To study the characteristics of a novel CWD strain in Swedish moose, we inoculated gene-targeted mice and followed the kinetics of disease until onset of clinical signs. Collected brain tissues were analyzed by western blot, histology features by immunohistochemistry and conformational stability by the presence of resistant PrP<sup>Sc</sup> after guanidine hydrochloride treatment. We show that Swedish moose CWD presents unique transmission properties, diagnosing clinical signs at ~90 days, one of the fastest progress to disease ever reported for a model of CWD. Other properties like conformational stability and histological features, were remarkably different from North American CWD and other strains in Europe, demonstrating the growing diversity of emergent CWD strains. Future studies will determine additional properties of this CWD strain which can shed light on the mechanisms of evolution of prion diseases in the wild and their zoonotic potential.

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## **136 Characterization of miRNA changes in Chronic Wasting Disease in Relation to Developing Early Detection Models**

Nicholas Heyer<sup>1</sup>, Mark Zabel<sup>1</sup>, Jason Bruemmer<sup>2</sup>

<sup>1</sup>Colorado State University, Fort Collins, USA. <sup>2</sup>National Wildlife Research Center, Fort Collins, USA

### **Abstract**

This project is to find a curated list of the most significant differences in miRNA in CWD infected cervids and a negative control to aid in subsequent longitudinal studies in transgenic mice. To do this both fecal samples and blood draws will be taken bi-weekly and RNA will be extracted and stored. Based on a previous paper by Jessy A. Slota et. al. in 2019 we expect to find 21 miRNAs that differ significantly and expect that the best predictive model will only include ~6 miRNAs after using an elastic net regression model and optimizing the penalization value. The results of this study serve to confirm the findings of the previously mentioned paper so that we have a set of punitive miRNA indicators of CWD, a pipeline for testing the effectiveness of them, and to confirm that fecal pellets are consistent with the blood samples. Subsequently, we will longitudinally assess at transgenic mice inoculated with CWD with regular sequencing of miRNA to see when or if it could be a safe, reliable, early, and non-lethal detection tool for CWD.

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## **138 Managing CWD Positive Ranched Cervid Herds in Saskatchewan.**

Petracek Harvey

Saskatchewan Cervid Alliance, Saskatoon , Saskatchewan, Canada

### **Abstract**

In 2018 Canadian Food Inspection Agency (CFIA) changed the approach to managing CWD in Canada. In areas where the disease is considered endemic, response is now considered a control operation rather than an eradication one. Producers who have been enrolled in Herd Certification Program are now the only ones eligible for indemnity or compensation provided they have met criteria for the program. Individuals may now also opt out of the process and continue to manage their herds under a quarantine from their individual province rather than depopulate.. Producers not enrolled in the program are no longer compensated nor are they ordered to depopulate, but are quarantined.

Since the change in policy over 12 herds have opted to operate under a herd management program in the province of Saskatchewan. The group includes elk, whitetail deer and mule deer. In addition to the provincial management program, the Saskatchewan Cervid Alliance has operated a pilot program to assess the use of ante-mortem testing. The program has involved the training of veterinarians

to correctly tissue sample animals using the RAMALT technique. As well the Prairie Diagnostics Lab in Saskatoon Saskatchewan was accredited through CFIA to provide analysis of these samples using Immuno-Histo-Chemistry. Four of the existing positive elk herds have utilized this program. Additionally, one of the whitetail deer herds has changed its breeding program to incorporate gene alleles believed to provide genetic resistance to CWD.

The use of RAMALT testing of live cervids plus genetic selection are part of six known tools for herd management of CWD infected animals. Additionally, biosecurity, nutrition, age management and vaccines under development are potentially available. The results over the 5 year period since the policy change have been encouraging. Elk which have been determined positive by RAMALT have been culled from the herds, euthenized and tested postmortem. To date every sample of approximately 150 which has been IHC positive has tested positive on post mortem.

The results of the testing program from 2019-22 have been thus far encouraging. It has allowed these operations to continue with CWD at a manageable level and has begun the ability to move tested animals to hunting preserves not contiguous to breeding operations. There have been some insights into how CWD spreads within a domestic herd which may not have been apparent previously. We are showing that herds are left to live and thus be monitored over extended time periods have yielded valuable information.

Our Association feels this project has been worthwhile and has provided a sense of optimism for a once feared and terminal outcome for stock and owners. We hope by sharing our outcomes, others will follow our pathway in management of this disease.

## **141 Intranasally Delivered Mesenchymal Stromal Cells Decrease Glial Inflammation Early in Prion Disease**

Arielle Hay, Amanda Latham, Genova Mumford, Amelia Hines, Sydney Risen, Mark Zabel, Julie Moreno

Colorado State University, Fort Collins, USA

### **Abstract**

Mesenchymal stromal cells (MSCs) are an intriguing avenue for the treatment of neurological disorders due to their ability to migrate to sites of neuroinflammation and respond to paracrine signaling in those sites by secreting cytokines, growth factors, and other neuromodulators. We potentiated this ability by stimulating MSCs with inflammatory molecules, improving their migratory and secretory properties. We investigated the use of intranasally delivered adipose-derived MSCs (AdMSCs) in combating prion disease in a mouse model. Early signs of prion disease include neuroinflammation, activation of microglia, and development of reactive astrocytes. Later stages of disease include development of vacuoles, neuronal loss, abundant aggregated prions, and astrogliosis. We demonstrate the ability of AdMSCs to upregulate anti-inflammatory genes and growth factors when stimulated with tumor necrosis factor alpha (TNF $\alpha$ ) or prion-infected brain homogenates. We stimulated AdMSCs with TNF $\alpha$  and performed biweekly intranasal deliveries of AdMSCs on mice that had been intracranially inoculated with mouse-adapted prions. At early stages in disease, animals treated with AdMSCs showed decreased vacuolization throughout the brain. Expression of genes associated with NF- $\kappa$ B and NLRP3 inflammasome signaling were decreased in the hippocampus. AdMSC treatment promoted a quiescent state in hippocampal microglia by inducing changes in both number and morphology. Animals that received AdMSCs showed a decrease in both overall and reactive astrocyte number, and morphological changes indicative of homeostatic astrocytes. Although this treatment did not prolong survival or rescue neurons, it demonstrates the benefits of MSCs in combatting neuroinflammation and astrogliosis.

## **143 Reduction of Chronic Wasting Disease Prion Seeding Activity following Digestion by Mountain Lions**

Chase Baune<sup>1</sup>, Lisa Wolfe<sup>2</sup>, Kristen Schott<sup>2</sup>, Karen Griffin<sup>2</sup>, Andrew Hughson<sup>1</sup>, Michael Miller<sup>2</sup>, Brent Race<sup>1</sup>

<sup>1</sup>Rocky Mountain Laboratory, Hamilton, USA. <sup>2</sup>Colorado Division of Parks and Wildlife, Fort Collins, USA

### **Abstract**

Chronic wasting disease (CWD) is a transmissible prion disease first observed in the 1960s in North America. This invariably fatal disease affects multiple cervid species in the wild and in captivity. In addition to the several known transmission pathways involving cervid host species, prions have been detected in the feces of crows and coyotes after consumption of experimentally spiked tissues. This raises questions about the role of cervid consumers in the perpetuation of CWD. Mountain lions have been shown to preferentially select CWD-infected prey and are also apparently resistant to infection. In this study, two captive mountain lions were fed ground mule deer muscle tissue spiked with brain-derived CWD prions, and lion feces were collected for 1 week afterward. The input brain and resulting fecal materials were analyzed using the highly sensitive real-time quaking-induced conversion (RT-QuIC) assay to quantify prion seeding activity. We recovered only 2.8 to 3.9% of input CWD prions after passage through the mountain lions' gastrointestinal tracts. Interestingly, CWD prions were shed only in the first defecation following consumption. Our data support the possibility that mountain lions feeding upon infected carcasses could excrete CWD prions in their feces over a short period of time but also suggest

that most of the ingested prions are eliminated or sequestered by this large predator.

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## **144 Disease surveillance of Chronic Wasting Disease in species of Cervids in Kansas and spatial and environmental factors affecting the epidemiological spread**

Joseph Mosley<sup>1</sup>, Emma Ragsdale<sup>1</sup>, Jared Bailey<sup>1</sup>, Olivea Moss<sup>1</sup>, Olivia Knowa<sup>1</sup>, Shane Hesting<sup>2</sup>, Levi Jaster<sup>2</sup>, Andrew Ross<sup>1</sup>, Terry Spraker<sup>3</sup>, Ram Raghavan<sup>4</sup>

<sup>1</sup>University of Missouri - Columbia, Columbia, USA. <sup>2</sup>Kansas Department of Wildlife and Parks, Emporia, USA. <sup>3</sup>Veterinary Diagnostic Laboratories - Colorado State University, Fort Collins, USA. <sup>4</sup>Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri - Columbia, Columbia, USA

### **Abstract**

Chronic Wasting Disease (CWD) is a fatal prion disease transmissible in captive and wild cervid populations, causing neurological degeneration, and physiological decay, such as weight loss and muscle coordination. From 2020-2023, we coordinated multi-method surveillance of wild cervids for CWD in partnership with the Kansas Department of Wildlife and Parks (KDWP). Samples of retropharyngeal lymph nodes (RPLN) and obex were collected from hunters, taxidermists, and biologists, who participated in the surveillance. Sampling kits were provided to each collector, which included printout instructions on how to obtain samples, what data to record (including age, sex, species, and location of harvest), and how to handle and ship specimens. In the 2020-21 season, we tested 1,907 samples (one sample per deer) within the state of Kansas. During the 2021-22 season, we tested 1,757 samples. In 2022-2023, we tested 1,816 samples for CWD. Of these specimens, 116 were detected to be positive for CWD through immunohistochemistry in 2020, 171 in 2021, and 187 in 2022. These were the positivity rates per year; in 2020 5.8% were positive, 9.7% in 2021, and 10.25% in 2022. Positivity rates for sex, age, and specific regions of Kansas also matched prior surveillance efforts. Our initial analyses of this epidemiological data revealed the presence of CWD in previously undetected counties in eastern Kansas and associations with environmental variables (soil, land cover). The next step in the analyses will be to apply models with the variable as covariates to inform further surveillance efforts and management steps.

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## **SPECIAL GUEST SPEAKER: Dr. Sylvie Bernstad- How is Europe dealing with emerging CWD?**

6:15 - 7:30pm Wednesday, 31st May, 2023

Plaza Ballroom



Sylvie Benestad ended her Doctoral studies at the University of Sport Sciences in Marseille (France) and the University of Medicine Oslo (Norway) in 1994. She has 25 years of experience working with prion diseases in animals, at the Norwegian Veterinary Institute in Oslo, both at diagnostic and research level. After working intensively with Nor98/atypical scrapie in sheep, she is since the first European case in Norway in 2016, focusing on CWD in the Nordic countries. She is leading one of the CWD reference laboratories designated by the WOA (ex OIE) since 2018.

Norwegian surveillance efforts were naturally boosted since the first CWD detection, with 164 000 tests performed since within seven years. The European Union launched a three- year program (2018-2020) for the 7 European countries having moose and/or reindeer, giving modest knowledge about the prevalence status in Europe.

It is demonstrated that the European CWD disease has multiple CWD types that challenge the knowledge acquired from CWD in North America at different levels, expanding the horizon of prion diseases in cervids.

Reindeer are now on the International Union for Conservation of Nature's Red List of Threatened Species UCNs list classified as vulnerable species, and management of the disease in Nordic cervids faces challenges.

## **Tom Thorne and Elizabeth Williams Memorial Award (WDA) and Beth Williams CWD Career Achievement Award**

7:30 - 8:00pm Wednesday, 31st May, 2023

Plaza Ballroom

### **Tom Thorne and Elizabeth Williams Memorial Award (through the WDA)**

The 2022 Tom Thorne and Elizabeth Williams Memorial Award will be presented at the 4th International CWD Symposium. Tom Thorne and Beth Williams were highly influential and revered members of the wildlife disease, conservation, and management community. Their dedication to wildlife health and conservation, many contributions to both WDA and AAWV, mentorship, scientific acumen, and friendship and love of life were an inspiration to all. Their tragic death in 2004 left a huge void. To help commemorate their lives and contributions to WDA and AAWV, a memorial award was created in their name.

This prestigious award is presented to an individual or group in acknowledgement of either an exemplary contribution or achievement combining wildlife disease research with wildlife management policy implementation or elucidating particularly significant problems in wildlife health. The recipient also receives a bronze of the black footed ferrets which commemorates the significant contributions they made to the health, conservation, and management of this endangered species.

For more information please see: <https://www.wildlifedisease.org/PersonifyEbusiness/About-Us/Awards/Tom-Thorne-and-Beth-Williams-Award>

### **Beth Williams CWD Career Achievement Award**

The Dr. Elizabeth (Beth) Williams career achievement award was developed to commemorate and celebrate her incredible life and to highlight the many contributions she made toward CWD research, management, and policy.

This award is presented to an individual who has demonstrated exemplary achievements in implementing CWD research, management, or policy. Nominations for this award should address the candidate's contributions or achievement in CWD management, research, and policy, and how those achievements have improved our understanding of CWD (e.g., ecology, biology, pathology, diagnostics, human dimensions, management, etc.) Nominations should also describe other notable contributions such as mentorship of students and early career professionals in the field of CWD, and extension and outreach to improve understanding of the disease in stakeholders and the public.

Dr. Terry Spraker was the recipient of this award

# Thursday, June 1<sup>st</sup>, 2023

## Young Professionals Breakfast

7:00 - 8:00am Thursday, 1st June, 2023

Plaza Court 2

Pre-Registration Required

This event will provide an opportunity for students and young professionals to interact with not only one another, but with individuals that may have upcoming open positions! Attendees will meet with like-minded professionals and learn about the latest advancements that will impact their careers. Young professional inspirational leaders will share insights, learnings, and failures from the early stages of their careers, with plenty of opportunities for attendees to ask questions. This is a unique opportunity for any students or Young Professionals that want the chance to grow their career and make the right connections that will propel their career forward.

## Plenary Talk: Dr. Glenn Telling- Characterizing the properties and host-range potentials of established and newly-emergent strains of chronic wasting disease prions

8:00 - 8:20am Thursday, 1st June, 2023

Plaza Ballroom



Glenn Telling P.h. D., Director, Prion Research Center (PRC)  
Professor of Microbiology, Immunology, and Pathology  
Colorado State University

Dr. Telling's background is in infectious diseases with specific training in the molecular genetics and cellular biology of DNA tumor viruses and prions. His involvement in prion research began with Nobel Laureate, Stanley Prusiner at UCSF. He was recruited to Imperial College, London at the height of the BSE/vCJD crisis to establish a research group in the newly-formed Medical Research Council (MRC) Prion Unit. Following this, he had a successful tenure at the Sanders Brown Center on Aging at the University of Kentucky Medical Center. In 2011, he was recruited to establish and direct the Prion Research Center at Colorado State University. His research as an independent investigator has been to understand the molecular basis of prion replication, species barriers, and strain inheritance, including how prions mutate and evolve in the absence of informational nucleic acids. During his career he has received uninterrupted NIH funding for a period covering 30 years. This includes multiple consecutive NIH awards, funding through the US Departments of Defense and Agriculture, and other UK and Canadian funding agencies. The consistent output of his lab during this time has exerted a powerful and sustained influence on the field. His research group is one of only a handful with the resources and expertise in transgenic, cell biological, biochemical, molecular genetic and in vitro approaches to study prion diseases, and we are widely considered to be among the leaders in the field. Of particular note, training scientists to perform research in this complicated field has been a top priority, and his protégés have obtained senior positions in leading academic and industrial research facilities in the US and Europe.

# Transmission

8:20 - 9:40am Thursday, 1st June, 2023

Plaza Ballroom

Moderator Dr. Tracy Nichols

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8:20 - 8:40am

## **Epidemiologic surveillance of prion disease among cervid hunters and people likely to have consumed venison contaminated with chronic wasting disease**

Joseph Abrams<sup>1</sup>, Ryan Maddox<sup>1</sup>, Lawrence Schonberger<sup>1</sup>, Arshi Chowdhury<sup>1</sup>, Lorna Will<sup>2</sup>, Suzanne Gibbons-Burgener<sup>2</sup>, Angela Maxted<sup>2</sup>, Natalie Marzec<sup>3</sup>, Clay Van Houten<sup>4</sup>, Courtney Tillman<sup>4</sup>, Brian Appleby<sup>5</sup>, Ermias Belay<sup>1</sup>

<sup>1</sup>Centers for Disease Control and Prevention, Atlanta, GA, USA. <sup>2</sup>Wisconsin Department of Health Services, Madison, WI, USA.

<sup>3</sup>Colorado Department of Public Health and Environment, Denver, CO, USA. <sup>4</sup>Wyoming Department of Health, Cheyenne, WY, USA.

<sup>5</sup>National Prion Disease Pathology Surveillance Center, Cleveland, OH, USA

### **Abstract**

Laboratory studies have raised the possibility that chronic wasting disease (CWD) in deer and elk could be transmissible to humans. In collaboration with state health departments, the US Centers for Disease Control and Prevention (CDC) has conducted multiple surveillance projects with the goal of identifying potential zoonotic transmission of CWD. Prion disease mortality surveillance has been conducted for people who purchased permits for hunting deer or elk from the Colorado Department of Wildlife (CDOW) since 1995 or from the Wyoming Game and Fish Department (WGFD) since 1996. Person-years from their first hunting year are counted for each hunter, and age-adjusted analyses are used to assess if hunters have elevated prion disease incidence compared to the general US population. In a separate project, CDC partners with the Wisconsin Department of Health Services (WDHS) to conduct prion disease surveillance on people who reported eating or having an intention to eat venison obtained from CWD-positive cervids. Prion deaths were identified through multiple cause of death data from state mortality records or the National Center for Health Statistics, or reported by the National Prion Disease Pathology Surveillance Center (NPDPS). These surveillance projects have not identified excess prion disease mortality among hunters in Colorado or Wyoming or any prion disease case in the Wisconsin study. However, given potentially long incubation periods, changes in the natural host or agent pathogenicity, and the continued spread of CWD, maintenance of vital surveillance projects are critical for the identification potential zoonotic transmission of CWD.

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8:40 - 9:00am

## **Efficient CWD-like transmission of splenic prions in cervidized transgenic mice: a probable diagnostic marker for CWD infection in humans**

Xu Qi, Liuting Qing, Manuel Camacho, Ignazio Cali, [Qingzhong Kong](#)

Department of Pathology, Case Western Reserve University, Cleveland, USA

### **Abstract**

Multiple in vitro CWD-seeded human PrP conversion experiments and some animal model studies indicate that the species barrier for CWD to human transmission can be overcome, but whether CWD prion can infect humans in real life remains controversial. The very limited understanding on the likely features of CWD infection in humans and the lack of a reliable marker for identification of acquired human CWD cases contribute to this uncertainty. We aim to establish a reliable diagnostic marker for CWD infections in humans should they occur.



We identified a couple of prion-positive spleens in humanized transgenic mice inoculated with some CWD isolates. Such experimentally generated splenic “humanized” CWD prions (termed eHuCWD<sup>SP</sup>) appear indistinguishable from prions in the brain of sCJDMM1 patients on Western blot. We compared eHuCWD<sup>SP</sup> with prions in the spleen from humanized mice infected with sCJDMM1 (termed sCJD<sup>SP</sup>) by bioassays in cervidized or humanized transgenic mice. Significantly, we found that eHuCWD<sup>SP</sup> can efficiently infect not only the humanized mice but also cervidized transgenic mice, and cervidized mice infected by eHuCWD<sup>SP</sup> produced prions and brain pathology that are practically identical to those of CWD-infected cervidized mice. In contrast, sCJD<sup>SP</sup>, similar to prions from sCJDMM1 patient brains, is poorly transmissible in the cervidized mice.

Our data demonstrate that high transmissibility with CWD features of splenic prions in cervidized transgenic mice is unique to acquired human CWD prions, and it may serve as a reliable marker to identify the first acquired human CWD cases.

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9:00 - 9:20am

## **Transmission studies of Chronic Wasting Disease to Squirrel monkeys, Cynomolgus macaques and transgenic mice expressing human prion protein**

Brent Race, Katie Williams, Chase Baune, Kimberly Meade-White, Andrew Hughson, Christina Orru, James Striebel, Bruce Chesebro

NIH Rocky Mountain Laboratory, Hamilton, USA

### **Abstract**

Chronic wasting disease (CWD) is a fatal prion disease that can infect deer, elk, and moose. CWD was first recognized in captive deer kept in wildlife facilities in Colorado from 1967 to 1979. Since that time CWD has spread significantly and is now reported in most states in the U.S.A, 3 Canadian provinces, South Korea, Norway, and Finland. It is currently unknown if humans are susceptible to CWD infection. Understanding the health risk associated with consuming meat and/or products from CWD-infected cervids is a critical human health concern. To date, cumulative research from many laboratories using in vitro and in vivo assays and epidemiologic studies suggests the risk of CWD transmission to humans is low.

In our laboratory, we have performed cross-species CWD transmission studies using two species of nonhuman primates (cynomolgus macaques and squirrel monkeys) and transgenic mice that over-expressed human prion protein (tg66) as transmission models. Non-human primates were inoculated by either the intracerebral or oral route with brain homogenates from CWD-infected deer and elk containing high levels of infectivity. Tg66 mice were inoculated intracerebrally with CWD, and two consecutive mouse passages were performed. Here we present a summary of the data from these three transmission studies including data from the ultrasensitive real-time quaking-induced conversion (RT-QuIC) assay, immunohistochemistry, immunoblotting, and bioassays in nonhuman primates and tg66 mice. Our data shows a strong species barrier limits CWD transmission to macaque monkeys and mice expressing human prion protein.

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9:20 - 9:40am

## **Are stable and highly zoonotic cervid prion strains possible?**

Manuel Camacho, Xu Qi, Liuting Qing, Sydney Smith, Jieji Hu, Wanyun Tao, Ignazio Cali, Qingzhong Kong

Department of Pathology, Case Western Reserve University, Cleveland, USA

### **Abstract**

Whether CWD prions can infect humans remains unclear despite the very substantial scale and long history of human exposure of CWD in many states or provinces of USA and Canada. Multiple in vitro conversion experiments and in vivo animal studies indicate that the CWD-to-human transmission barrier is not unbreakable. A major long-term public health concern on CWD zoonosis is the emergence of highly zoonotic CWD strains. We aim to address the question of whether highly zoonotic CWD strains are possible.

We inoculated a few sCJD brain samples into cervidized transgenic mice (Tg12), which were intended as negative controls for bioassays of brain tissues from sCJD cases who had hunted or consumed venison from CWD-endemic states. Some of the Tg12 mice became infected, resulting in a “cervidized” CJD strain that we termed CJD<sup>EIkPrP</sup>. CJD<sup>EIkPrP</sup> was further examined by serial passages in humanized or cervidized mice. We observed 100% transmission of the original CJD<sup>EIkPrP</sup> in transgenic mice expressing human PrP.

We passaged CJD<sup>EIkPrP</sup> two more times in the Tg12 mice. We found that such second and third passage CJD<sup>EIkPrP</sup> prions retained 100% transmission rate in the humanized mice. In contrast, we and others found zero or poor transmission of natural elk CWD isolates in humanized mice, despite that the natural elk CWD isolates and CJD<sup>EIkPrP</sup> share the same elk PrP sequence. Our data indicate that highly zoonotic cervid prion strains are not only possible but also can be stably maintained in cervids.

## **BREAK**

9:40 - 10:00am Thursday, 1st June, 2023  
Plaza Exhibit Foyer

## **Transmission**

10:00 - 11:40am Thursday, 1st June, 2023  
Plaza Ballroom

Moderator Dr. Tracy Nichols

10:00 - 10:20am

## **Transmission of prion infectivity from CWD-infected macaque tissues to rodent models demonstrates the zoonotic potential of chronic wasting disease.**

Samia Hannaoui<sup>1,2</sup>, Ginny Cheng<sup>1,2</sup>, Wiebke Wemheuer<sup>3</sup>, Walter Schulz-Schaeffer<sup>3</sup>, Sabine Gilch<sup>1,2</sup>, Hermann Schatzl<sup>1,2</sup>

<sup>1</sup>University of Calgary, Calgary, Canada. <sup>2</sup>Calgary Prion Research Unit, Calgary, Canada. <sup>3</sup>Institute of Neuropathology, Medical Faculty, Saarland University, Homburg/Saar, Germany

### **Abstract**

We provide evidence by transmission experiments to different transgenic mouse models and bank voles that *Cynomolgus* macaques inoculated via different routes with CWD-positive cervid tissues harbor infectious prions. We used tissue materials from macaques inoculated with CWD to inoculate transgenic mice overexpressing cervid PrP<sup>C</sup> followed by transmission into bank voles. We used RT-QuIC, immunoblot and PET blot analysis to assess brains, spinal cords, and tissues of the gastrointestinal tract (GIT) for the presence of prions. Our results show that macaque materials induced clinical disease in transgenic mice with low attack rates. Clinical mice did not display PrP<sup>Sc</sup> in immunoblot, but showed low-levels of prion seeding activity. Further transmission into bank voles led to a 100% attack rate with typical PrP<sup>Sc</sup> signature in immunoblot, and high-level prion seeding activity in brain, spinal cord and GIT tissues. Second passage studies led to 100% attack rate in voles inoculated with brain, spinal cord and small intestine material from first round animals, with shortened survival times indicating adaptation in the new host. This shows that prions detected in GIT tissues are infectious and transmissible. Further passage to cervidized mice revealed transmission with a 100% attack rate. Our findings demonstrate that macaques, considered the best model for the zoonotic potential of prions, were infected upon CWD challenge, including the oral one. The disease manifested as atypical in macaques and initial transgenic mouse transmissions, but with infectivity present at all times, as unveiled in the bank vole model with an unusual tissue tropism.

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10:20 - 10:40am

## Detection of infectious prions in tissues of feral hogs

PAULINA SOTO<sup>1,2</sup>, Francisca Bravo-Risi<sup>3,4</sup>, Rebeca Benavente<sup>3</sup>, Michael J. Bodenchuk<sup>5</sup>, Patrick Whitley Whitley<sup>6</sup>, Clint Turnage<sup>6</sup>, Tracy A Nichols<sup>7</sup>, Terry Spraker, Vienna R Brown<sup>6</sup>, Rodrigo Morales<sup>1,2</sup>

<sup>1</sup>Department of Neurology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, USA.

<sup>2</sup>Universidad Bernardo O'Higgins, Santiago, Chile. <sup>3</sup>Department of Neurology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, Chile. <sup>4</sup>Universidad Bernardo O'Higgins, Santiago, Chile. <sup>5</sup>United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, San Antonio, USA. <sup>6</sup>USDA APHIS Wildlife Services, Fort Collins, USA.

<sup>7</sup>Veterinary Services Cervid Health Program, United States Department of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, USA

### Abstract

Chronic wasting disease (CWD) is the prion disease with the greatest potential for spreading, affecting at least seven cervid species. An essential feature of prions lies in their ability to infect some species and not others. This phenomenon, known as a species barrier, seems to be largely dictated by the similarities between the donor (infectious) and recipient prion protein (PrP) sequences. Considering this, some potentially susceptible carnivores are proposed to act as vectors of CWD transmission as they may get infected.

Alternatively, predators or scavengers may not get infected but spread infectious particles after they cross their digestive tracts. This project aims to identify the presence of infectious prions in feral hogs living in CWD-endemic areas. To do this, PMCA seeding activity was analyzed on feral hogs tissues such as the brain and retropharyngeal and submandibular lymph nodes using homologous pig PrP substrate or heterologous deer PrP substrate. We further injected selected feral hogs tissues into mice expressing the cervid and porcine versions of the prion protein to assess their potential to transmit disease. Our results show positive *in vitro* PrPSc detection using porcine and cervid substrates. The considerably higher detection using cervid substrate suggests that although feral hogs are exposed to CWD prions, disease transmission is inefficient. Bioassays confirmed these results and demonstrated that the infectivity carried by feral hogs is not enough to induce disease. In summary, these results suggest that feral hogs may play a role in disseminating CWD prions across the landscape.

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10:40 - 11:00am

## Chronic wasting disease *in utero* transmission in free-ranging white-tailed deer

Audrey Sandoval<sup>1</sup>, Amy Nalls<sup>1</sup>, Erin McNulty<sup>1</sup>, Nathaniel Denkers<sup>1</sup>, Zoe Olmstead<sup>1</sup>, Ethan Barton<sup>2</sup>, Jennifer Ballard<sup>3</sup>, Daniel Grove<sup>4</sup>, Jeremy Dennison<sup>5</sup>, Natalie Stilwell<sup>2</sup>, Christopher Cleveland<sup>2</sup>, James Crum<sup>6</sup>, Mark Ruder<sup>2</sup>, Candace Mathiason<sup>1</sup>

<sup>1</sup>Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, USA. <sup>2</sup>Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia, Athens, USA.

<sup>3</sup>Arkansas Game and Fish Commission, Little Rock, USA. <sup>4</sup>University of Tennessee Extension, Nashville, USA. <sup>5</sup>Tennessee Wildlife Resources Agency, Jackson, USA. <sup>6</sup>Wildlife Resources, West Virginia Division of Natural Resources, Elkin, USA

### Abstract

The transmission of chronic wasting disease (CWD) within cervid populations has been largely attributed to contact with infectivity present in bodily secretions via direct (animal-to-animal) or indirect (shed prions in the environment) contact, i.e. horizontal transmission. Previous studies in free-ranging white-tailed deer (WTD) and Rocky Mountain elk, and experimental studies in Reeves' muntjac deer have demonstrated that mother-to-offspring transmission is another likely source of infection, i.e. vertical transmission. To expand on this research, we investigated the role *in utero* transmission plays in free-ranging WTD with naturally acquired infections from three states in the southeastern U.S. Maternal and *in utero*-derived fetal tissues (n=56) were harvested from healthy appearing dams (n=31) in CWD endemic areas of Arkansas, Tennessee, and West Virginia as well as one state with no known CWD infections (Georgia). Tissues were assessed for amyloid seeding activity (prions) using real time quaking induced conversion (RT-QuIC), immunohistochemistry (IHC), protein misfolding cyclic amplification (PMCA), and/or iron-oxide bead extraction (IOB). To determine the presence of infectivity in these samples, Tg5037 mouse bioassay was initiated to assess maternal and fetal tissues. We found CWD prions in the maternal reproductive tract (uterus, placentomes and amniotic fluids) and fetal tissues by RT-QuIC analysis; suggesting fetal exposure prior to parturition. Tg5037 mouse bioassay revealed the presence of infectivity in both maternal reproductive tissues and fetal tissues. This study supports earlier findings, and now expanded geographical distribution of CWD mother-to-offspring transmission in white-tailed deer, providing further understanding of the facile and efficient transmission of CWD.

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11:00 - 11:20am

## **Wisc-1 chronic wasting disease prions are selecting for wild white-tailed deer that express the 96S prion protein polymorphism**

Allen Herbst<sup>1</sup>, Jay Schneider<sup>1</sup>, Aaron Lomax<sup>1</sup>, Stuart Lichtenberg<sup>2</sup>, Alison Ketz<sup>3</sup>, Daniel Walsh<sup>4</sup>, Daniel Storm<sup>5</sup>

<sup>1</sup>US Geological Survey, National Wildlife Health Center, Madison, USA. <sup>2</sup>Dept. of Soil Sciences, University of Wisconsin-Madison, Madison, USA. <sup>3</sup>Wisconsin Cooperative Wildlife Research Unit, University of Wisconsin-Madison, Madison, USA. <sup>4</sup>US Geological Survey, Montana Cooperative Wildlife Research Unit, Missoula, USA. <sup>5</sup>Wisconsin Department of Natural Resources, Eau Claire, USA

### **Abstract**

Chronic wasting disease (CWD) was first detected in southwestern Wisconsin in 2001. A 2001-2004 survey of the prion protein isoforms present in Wisconsin white-tailed deer identified a putative resistance polymorphism, 96S. 96S has a serine instead of a glycine (96G) at amino acid residue 96 of the white-tailed deer prion protein. We hypothesized that CWD is selecting for deer expressing the 96S isoform of the prion protein. We performed DNA sequencing of the prion protein gene from 877 deer from southwestern Wisconsin in the years 2017-2020. From 2004 to 2020, the percentage of deer containing at least one allele encoding the 96S prion proteoform increased from 28% to 62%. The percentage of deer that were homozygous (96SS) increased from 2% to 13.3%. CWD was rare in 96SS deer (3%), but prevalent in 96GS and 96GG deer, 32% and 50% respectively. We conclude that the 96S variant of the white-tailed deer offers partial resistance to the Wisc-1 CWD prion strain responsible for the outbreak in Wisconsin and that CWD is selecting for deer that express prion proteins with the 96S polymorphism.

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11:20 - 11:40am

## **A comparison of CWD and scrapie in white-tailed deer and sheep**

Justin Greenlee<sup>1</sup>, Zoe Lambert<sup>1</sup>, Jifeng Bian<sup>1</sup>, M Heather West Greenlee<sup>2</sup>

<sup>1</sup>USDA, ARS, National Animal Disease Center, Ames, USA. <sup>2</sup>Iowa State University, Ames, USA

### **Abstract**

The US No. 13-7 scrapie isolate has a 100% attack rate in white-tailed deer (WTD). Samples from the brainstems and lymph nodes of affected deer are difficult to discern from those of deer with CWD by western blot, though samples from the cerebrum or retina of WTD with scrapie retain a molecular profile similar to the scrapie inoculum. In contrast, transmission of CWD prions from WTD to sheep does not readily occur. Only a single sheep had evidence of PrPSc after oronasal inoculation, and results of further passage in sheep were negative. To assess potential transmission of scrapie from WTD back to sheep after oronasal inoculation, we used samples of cerebrum and brainstem from scrapie affected WTD. A subset of sheep developed clinical disease. The first sheep to develop disease were inoculated with material from the WTD cerebrum. Two VRQ/VRQ sheep at 29 months post inoculation (MPI) and one ARQ/ARQ sheep at 48 MPI. Only a single VRQ/VRQ sheep inoculated with deer brainstem material developed clinical disease after 66 MPI. Assessment of brains from affected sheep by immunohistochemistry and stability assays indicate that the passage of scrapie through deer results in a phenotype switch: the staining characteristics and stability are different than the original No. 13-7 inoculum and similar to another strain of scrapie called x124. This work demonstrates that deer are readily infected with scrapie prions from sheep and passage back to sheep resulted in altered strain properties.

## **LUNCH**

11:40am - 1:00pm Thursday, 1st June, 2023

Local restaurants can be found at

[www.visitDenver.com](http://www.visitDenver.com)

# Intervention/Prevention

1:00 - 2:00pm Thursday, 1st June, 2023

Plaza Ballroom

Moderator Dr. Patricia Klein

1:00 - 1:20pm

## **Rationally designed, structure-based vaccine candidates targeting Chronic Wasting Disease**

Andrew Fang<sup>1</sup>, Xinli Tang<sup>1</sup>, Brian Tancowny<sup>1</sup>, Glenn Telling<sup>2</sup>, Holger Wille<sup>1</sup>

<sup>1</sup>Centre for Prions and Protein Folding Diseases, University of Alberta, Edmonton, Canada. <sup>2</sup>Prion Research Center, Colorado State University, Fort Collins, USA

### **Abstract**

Chronic wasting disease (CWD) is the most contagious prion disease, affecting free-ranging and captive cervids, including deer, elk, and moose. No cures or prophylactic vaccines exist for any prion disease including CWD, resulting in an invariably fatal outcome for an infected host.

Based upon the theoretical four-rung  $\beta$ -solenoid model of PrP<sup>Sc</sup>, our lab has developed a protocol using a modified fungal prion protein as an innocuous scaffold for the rational design of structure-based vaccine candidates targeting CWD. The alternating position of amino acid side chains within the  $\beta$ -strands (inwards versus outwards facing) was used to strategically place predicted surface residues of PrP<sup>Sc</sup> onto the surface of the scaffold. The resulting vaccine was then used to immunize mice that spontaneously developed a genetic prion disease. Vaccine immunized mice were able to remain healthy for significantly longer (448 $\pm$ 39 days) than unimmunized (177 $\pm$ 17 days) and scaffold immunized controls (161 $\pm$ 27 days). Sera were collected prior to the priming dose and 2 weeks following each boost for pre- and post-immune sera. The post-immune sera showed a markedly increased antibody titre over unimmunized mice and were able to preferentially recognize prion-infected brain samples versus uninfected controls.

A monoclonal antibody derived from the spleens of vaccine immunized mice was shown to preferentially recognize human and animal prion disease strains over their non-infectious counterparts using competition ELISAs. Future directions include vaccine optimizations to elicit a mucosal immunoglobulin A response for the vaccination of cervids against oral CWD exposure (See also VanWick et al. abstract).

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1:20 - 1:40pm

## **Development and Evaluation of CWD Biosecurity Practices using a Risk Assessment Approach by Farmed Cervid Producers**

Scott Wells<sup>1</sup>, Michelle Schultze<sup>1</sup>, Tiffany Wolf<sup>1</sup>, Marc Schwabenlander<sup>1</sup>, Linda Glaser<sup>2</sup>, Courtney Wheeler<sup>2</sup>, Amy Horn-Delzer<sup>3</sup>, Melissa Graham<sup>3</sup>, Alex Hamberg<sup>4</sup>, David Zellner<sup>4</sup>

<sup>1</sup>University of Minnesota, St. Paul, USA. <sup>2</sup>Minnesota Board of Animal Health, St. Paul, USA. <sup>3</sup>Wisconsin Dept of Agriculture, Trade and Consumer Protection, Madison, USA. <sup>4</sup>Pennsylvania Dept of Agriculture, Harrisburg, USA

### **Abstract**

Due to variability in transmission pathways through which CWD may be introduced to cervid operations, cervid producers need to develop and implement appropriate biosecurity practices based on recognition of the most important pathways of infection for their own operations. Objectives of this USDA-APHIS funded project (2021-2023) included 1) develop an on-farm CWD biosecurity assessment tool for cervid producers and their veterinarians to assess risk of CWD introduction to the operation, 2) develop CWD biosecurity educational resources to support implementation of biosecurity on cervid operations, and 3) evaluate the impact of implementing

biosecurity program on cervid operations that implement the biosecurity risk assessment program. Based on published literature and other available information, we created a CWD Biosecurity Assessment for on-farm use to provide cervid producers with a systematic process to identify and prioritize risks of CWD introduction to their operation from each transmission pathway. After input from a pilot sample of cervid producers to allow refinement of the assessment tool, we collected information from a sample of volunteer cervid producers in Minnesota, Pennsylvania, and Wisconsin. We also created educational materials for cervid producers using a web-based format to support assessment use, which included real-world scenarios. Results showed that this assessment tool was useful to identify and prioritize CWD introduction risks to different cervid operations. Participating cervid producers provided positive feedback about use of the CWD Biosecurity Assessment to identify areas of potential CWD introduction that had not been previously considered to allow herd biosecurity enhancements.

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1:40 - 2:00pm

## Genomic Predictions For Differential Susceptibility to Chronic Wasting Disease in Farmed U.S. White-tailed Deer

[Christopher Seabury](#)<sup>1</sup>, Tracy Nichols<sup>2</sup>

<sup>1</sup>Texas A&M University, College Station, USA. <sup>2</sup>USDA APHIS Veterinary Services, Fort Collins, USA

### Abstract

Despite advanced management practices, diagnostic surveillance, and depopulation of positive herds, the geographic expansion of chronic wasting disease (CWD) in U.S. white-tailed deer (*Odocoileus virginianus*; hereafter WTD) remains largely unabated. Therefore, a critical need exists to identify novel strategies that reduce the prevalence of CWD. Herein, we deploy a custom single nucleotide polymorphism (SNP) array to perform the largest genome-wide association study for CWD in farmed U.S. WTD to date; thereby confirming *PRNP* codon 96 as the largest-effect region of the WTD genome ( $P$ -value  $\leq 5.99e-13$ ; Proportion of Variance Explained  $\geq 0.032$ ). However, beyond *PRNP*, 25 significant SNPs ( $P$ -value  $\leq 5e-05$ ) also were detected, thereby implicating  $\geq 25$  unique positional candidate genes; many of which have been directly implicated in aspects Alzheimer's, Parkinson's, and various prion diseases. Our genome-wide association analyses unequivocally indicate that differential susceptibility to CWD and variation in natural disease progression have trait architectures comprised of very few moderate or large-effect genetic components, and many small-effect genetic components; which is consistent with most polygenic traits. Interestingly, genomic relationship matrix heritability estimates were high for both differential susceptibility to CWD ( $h^2 = 0.611 \pm 0.056$ ), and for variation in natural disease progression ( $h^2 = 0.589 \pm 0.069$ ); indicating that the majority of the variation in these traits can be explained by genetic differences. Likewise, heritability estimates produced for differential susceptibility to CWD on the liability scale, with adjustment for ascertainment of the case samples (using the weighted mean prevalence) were also high ( $h^2 \geq 0.857 \pm 0.082$ ); only further confirming that the majority of the variation in CWD susceptibility can be explained by genetic differences. To investigate the potential impact of deploying genomic predictions as a management-level decision support tool intended to reduce the prevalence of CWD in farmed U.S. WTD, we used genomic best linear unbiased prediction in conjunction with k-fold cross validation ( $k = 3$ ;  $k = 5$ ) and random sampling across 50 iterations for multiple models. Across all model fits and iterations, mean genomic prediction accuracies were high ( $\geq 0.81$ ). For comparison, we also performed leave-one-out cross-validation; which produced similar results related to genomic prediction accuracy. To further evaluate the utility of a genomic prediction program for CWD management-level decision support, a blind validation was administered by USDA-APHIS. The mean sensitivity of the blind genomic predictions was  $\geq 0.87$ . Collectively, our analyses indicate that WTD breeding programs utilizing genomic predictions for CWD management-level decisions such as genetic improvement through selective breeding, and/or the targeted removal of moderate and highly susceptible WTD, would be expected to reduce the prevalence of CWD.

## Human Dimensions

2:00 - 3:00pm Thursday, 1st June, 2023

Plaza Ballroom

Moderator Dr. Jennifer Malmberg

2:00 - 2:20pm

# Economic costs of chronic wasting disease in the United States

Scott Chiavacci

United States Geological Survey, Reston, USA

## Abstract

Cervids are economically important to a wide range of stakeholders and rights holders in the United States. The continued expansion of CWD poses a direct and indirect threat to state and federal government agency operations and cervid related economic activity. However, the scale of this disease's direct economic costs is largely unknown. I synthesized existing data to estimate CWD's costs within the continental United States. Federal government agencies collectively spent over \$284.1 million on CWD between 2000 and 2021. In one fiscal year (2020), state natural resources agencies and state agriculture/animal health agencies spent over \$25.5 million and \$2.9 million, respectively, on CWD-related work. Natural resources agencies in states with known CWD cases spent over 8 times as much on CWD as agencies from states with no known cases. The farmed cervid industry spent at least \$307,950 on CWD sampling in 2020, though a lack of available data prevented a complete assessment of costs to this industry. Based on limited data, CWD's economic effects on the hunting industry (i.e., outfitters and guides, companies leasing land to cervid hunters), may be negligible at this time. Overall, however, the realized economic costs of CWD appear considerable, and it is likely that the number of stakeholders financially affected by this disease will continue to grow.

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2:20 - 2:40pm

## Applying Theories of Change to CWD Management

Nicholas Cole

US Geological Survey, Fort Collins, USA

## Abstract

Chronic Wasting Disease (CWD) is a fatal, neurological degenerative disease that has infected cervid species (deer, elk, moose, reindeer) in North America, Northern Europe, and Korea and has no known cure. A transmissible spongiform encephalopathy (TSE), abnormal prions proliferate within infected cervids and pass directly between individuals or persist in the environment and result in further spread of the disease. CWD exists heterogeneously across North America with some areas having identified outbreaks going back to the mid-1980's and others having not identified outbreaks yet. A great deal of investigation has been conducted across many disciplines and has culminated in comprehensive guidance in managing CWD. Organizations like the Association of Fish and Wildlife Agencies have developed highly effective descriptions of best management practices that inform and list an array of strategies for managing chronic wasting disease. These best management practices are grounded in current and effective scientific information. CWD management in North America should be viewed as a complex system and efforts to map the broad interactions between social and ecological factors are needed to support existing best management practices. Through a virtual multi-day workshop series, we developed theories of change (TOC) to actively integrate social science with CWD biological management where interventions are theorized to have a positive impact on the conservation target. The TOCs were developed following the Conservation Standards Framework using a CWD specific social-ecological systems (SES) model of CWD management. This presentation will outline the challenges surrounding CWD management, how to create SES models and TOCs using the Conservation Standards, our process using an expert elicitation workshop, and how generic theories of change can be used to improve CWD management outcomes and to adapt specific management best practice using social science inquiry.

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2:40 - 3:00pm

## **Expanding CWD Educational Materials**

Miranda Huang, Steve Demarais, Bronson Strickland

Mississippi State University, Starkville, USA

### **Abstract**

As chronic wasting disease (CWD) spreads, more and more people are forced to come to terms with a disease they've never encountered and that is unlike familiar wildlife health issues. Hunter uncertainty may decrease hunting participation and willingness to engage in management actions aimed at limiting the spread of CWD. Agencies have produced educational materials for traditional media (newspapers, radio, magazines), but effective messaging via social media (Instagram, Facebook, etc.) has been limited. However, social media is a significant source of information for a large percentage of Americans. In this talk, we'll discuss the 12 animated videos we produced that cover the basics of chronic wasting disease in 2- to 3-minute segments. These videos were a collaboration between the Mississippi State University Deer Lab and 10 partners to generate educational materials that can be used broadly and disseminated over social media to help the public understand this new disease affecting deer herds across North America.

## **BREAK**

3:00 - 3:20pm Thursday, 1st June, 2023  
Plaza Exhibit Foyer

## **Human Dimensions Continued**

3:20 - 4:40pm Thursday, 1st June, 2023  
Plaza Ballroom

Moderator Dr. Jennifer Malmberg

3:20 - 3:40pm

## **Perceived Risks and Agency Trust Associated with Chronic Wasting Disease Over Time: A Meta Analysis**

Jerry Vaske<sup>1</sup>, Craig Miller<sup>2</sup>

<sup>1</sup>Colorado State University, Fort Collins, USA. <sup>2</sup>University of Illinois, Champaign, USA

### **Abstract**

Chronic wasting disease (CWD) is a transmissible neurological disease among Cervids. This meta-analysis examined changes in perceived risks to deer and humans, and perceived agency trust associated with CWD over time and across 12 studies. Perceived risk is a belief that a person has been exposed to a hazard like CWD. Trust refers to the confidence individuals have in officials assigned to control the disease. A 2004 regional study provided deer hunter data in seven states (Colorado, Nebraska, North Dakota, South Dakota, Utah, Wisconsin, Wyoming). Deer hunter data were also obtained from Colorado (2005), Illinois (2012, 2022), Kansas (2020), and North Dakota (2021). The independent variable was the difference in years between when the disease was discovered in the state and the when the study was conducted (range=2 to 38 years). There were three categories of multiple-item dependent variables: risk to deer, risk to humans, and agency trust. The total sample size was 23,205 (average response rate=44%). Results indicated that perceived risks to both deer and humans declined the longer the disease had been in the state. The risk to deer, however, dropped sharply during early years after the discovery and then declined gradually over the other years. Risk to humans gradually declined form



year 2 through year 19 differences, and then dropped sharply. Agency trust evaluations were positive, except for the first yearly differences. Trust increased dramatically from the 4-year to the 5-year difference and then leveled off. These results highlight how risks and agency trust change over time.

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3:40 - 4:00pm

## **Hunter response to changes in Chronic Wasting Disease (CWD) prevalence in Alberta**

Qin Xu<sup>1</sup>, Vic Adamowicz<sup>1</sup>, Marty Luckert<sup>1</sup>, John Pattison-Williams<sup>1</sup>, Maria Dobbin<sup>2</sup>, Anne Hubbs<sup>3</sup>, Margo Pybus<sup>2,4</sup>, Evelyn Merrill<sup>2</sup>

<sup>1</sup>Department of Resource Economics and Environmental Sociology, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, Canada. <sup>2</sup>Department of Biological Sciences, University of Alberta, Edmonton, Canada. <sup>3</sup>Alberta Fish and Wildlife Stewardship, Government of Alberta, Rocky Mountain House, Canada. <sup>4</sup>Alberta Fish and Wildlife Stewardship, Government of Alberta, Edmonton, Canada

### **Abstract**

Chronic Wasting Disease (CWD) has the potential to diminish deer populations and affect various segments of society including recreational hunters. Hunter harvest programs can be a cost-effective and socially acceptable approach to CWD management. However, there is limited information on hunter behavioral responses to CWD and whether hunters will maintain hunting efforts in areas where CWD prevalence increases. Our study estimates the effect of CWD prevalence, mandatory CWD-testing requirements, and other factors on hunter applications for draw licenses for mule deer (*Odocoileus hemionus*) in Alberta from 2006-2022 as the first step in evaluating whether hunter harvest can be used for CWD management over time. We use application data aggregated within 37 Wildlife Management Units (WMUs) in two-way fixed effects models to identify how CWD prevalence levels influence the number of draw applications. Preliminary results indicate that applications for mule deer decline when CWD prevalence rates increase. For example, in a given year, the applications for antlered mule deer licenses are estimated to decline by 6.6% if CWD prevalence rates increase from 10% to 20%. Our findings suggest there may be limitations regarding the extent to which hunters may be willing to participate in CWD management over time.

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4:00 - 4:20pm

## **Identifying the Motivations and Barriers of Landowners to Participating in Targeted Culling to Manage Chronic Wasting Disease (CWD)**

Jason Isabelle<sup>1</sup>, Jasmine Batten<sup>2</sup>, Phil Seng<sup>3</sup>, Matt Harlow<sup>3</sup>, Rachel Menale<sup>3</sup>

<sup>1</sup>Missouri Department of Conservation, Columbia, USA. <sup>2</sup>Wisconsin Department of Natural Resources, Madison, USA. <sup>3</sup>DJ Case & Associates, Mishawaka, USA

### **Abstract**

Since first detecting chronic wasting disease (CWD) in the free-ranging white-tailed deer population in 2012, the Missouri Department of Conservation has implemented targeted culling to slow disease spread. Culling is one of few interventions with evidence of efficacy yet is often met with resistance and lack of stakeholder support. As such, identifying the motivations and barriers of landowners to participating in culling is key to addressing participation deficiencies and fostering landowner engagement in CWD management. We conducted 12 focus group meetings in Missouri during 2022 with landowners who owned property near where CWD has been detected. Motivations for participating in culling included a sense of civic responsibility to people and/or the deer population, additional hunting opportunity, food, convenience of the culling program, and positive feelings about contributing to CWD management. Barriers or deterrents to participation included misunderstanding about CWD and the objectives of culling, perception of low deer density, reluctance to relinquish what landowners felt was management control of their land during culling, distain for culling methods (e.g., night shooting, baiting), and objections about when culling occurred (i.e., winter, after deer season). Landowner participation in culling could likely be increased by communicating more effectively about CWD and the objectives of culling to combat misinformation, providing additional opportunity during the regular deer season enabling hunters to play a more active role in disease management, considering changes to culling methodology (e.g., not field dressing deer on site), and working to align culling goals with landowner deer management goals when possible.

4:20 - 4:40pm

## **Integrating social science to make better decisions for cervid carcass management**

Margaret C. McEachran<sup>1,2</sup>, Jonathan D. Cook<sup>2</sup>, Richard E. W. Berl<sup>2</sup>, Graziella DiRenzo<sup>3,1</sup>, Evan H. Campbell Grant<sup>4</sup>, Michael C. Runge<sup>2</sup>

<sup>1</sup>Department of Environmental Conservation, University of Massachusetts, Amherst, MA, USA. <sup>2</sup>Eastern Ecological Science Center, United States Geological Survey, Laurel, MD, USA. <sup>3</sup>Massachusetts Cooperative Fish and Wildlife Research Unit, United States Geological Survey, Amherst, MA, USA. <sup>4</sup>Eastern Ecological Science Center, United States Geological Survey, Turner's Falls, MA, USA

### **Abstract**

Despite decades of work to better understand the biology and ecology of chronic wasting disease (CWD), successful strategies for mitigating its effects in free-ranging cervids remain elusive, for several reasons. First, there remain substantial uncertainties surrounding CWD transmission and other disease dynamics. Second, human behaviors (e.g., hunting, feeding, and carcass disposal) also influence disease risk in captive and free-ranging cervid systems. Third, decision-makers face impediments such as limited resources, contested jurisdictional boundaries, and multiple competing objectives. To address these challenges, we present a decision-analytical framing that integrates risk and social dimensions modeling and discuss how it can enhance existing strategies for evidence-based management of CWD. Hunter-mediated carcass movement represents a potential route for long-lasting environmental contamination by infectious prions, and subsequently much effort has been spent on hunter education campaigns, carcass dumpster programs, and carcass movement regulations. Little is known, however, about the success of translating these efforts to human behavior change, and the relative risk reduction that these behavior changes accomplish. We are partnering with deer program managers to pilot a decision analytical approach for management of hunter-killed cervid carcasses. In this talk, we outline our approach: using predictive social science in a decision analytical framework to characterize the CWD risk landscape, forecasting impacts of potential management alternatives on CWD program goals, and evaluating tradeoffs for carcass management objectives.

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## **POSTER SESSION & HAPPY HOUR**

4:40 - 6:00pm Thursday, 1st June, 2023  
Plaza Exhibit Foyer

Posters listed on the poster session for Wednesday evening

Two drink tickets in registration packet

# State/Provincial Wildlife Management Roundtable

6:00 - 7:30pm Thursday, 1st June, 2023

Plaza Ballroom

State and provincial natural resource management agencies face a myriad of challenges responding to Chronic Wasting Disease (CWD). In this moderated roundtable discussion, scientists and management experts from affected jurisdictions will discuss challenges and opportunities related to CWD management, with a glimpse to what the future of CWD management might hold.

Panel:

Margo Pybus, Alberta Fish and Wildlife

Mike Tonkovich, Ohio Department of Natural Resources

Justin Binfet, Wyoming Game and Fish Department

Mary Wood, Colorado Parks and Wildlife

Cory Gray, Arkansas Game and Fish Commission

William McKinley, Mississippi Department of Wildlife, Fisheries, and Parks

# Friday, June 2<sup>nd</sup>, 2023

***Registration 8:00 AM - 10:00 AM***

**Plenary Speaker: Dr. Jason Bartz- CWD Research: Past, Present and Future**

8:00 - 8:20am Friday, 2nd June, 2023  
Plaza Ballroom



Jason C. Bartz, Ph.D.  
Professor and Chair  
Department of Medical Microbiology & Immunology  
Associate Dean of Academic & Faculty Affairs  
School of Medicine, Creighton University

Dr. Bartz has been investigating prions for over 30 years. Dr. Bartz's research has investigated interspecies transmission, pathogenesis, and the biology of prion strains. Dr. Bartz's group has developed novel whole animal and in vitro models of prion disease and, more importantly, the results from these systems have changed the paradigm of how a protein-only infectious agent can perform complex biological tasks. More recently, Dr. Bartz has expanded his studies to include the interaction of prions with the environment by leveraging his expertise in prion biology with collaborators in environmental engineering resulting in fundamental discoveries on how prions interface with the environment influences the biology of prion disease.

# CWD Epidemiology

8:20 - 8:40am Friday, 2nd June, 2023

Plaza Ballroom

Moderator Dr. Glenn Telling

## Risky Business: Relating Probability of Direct Contact with Risk of Chronic Wasting Disease

Maria Dobbin<sup>1</sup>, Peter Smolko<sup>2</sup>, Laurens Put<sup>1</sup>, [Evelyn Merrill](#)<sup>1</sup>

<sup>1</sup>Biological Sciences, University of Alberta, Edmonton, Canada. <sup>2</sup>Department of Applied Zoology and Wildlife Management, Technical University in Zvolen, Zvolven, Slovakia

### Abstract

Chronic wasting disease (CWD) is a fatal, prion disease of cervids that was first detected in Alberta in 2005. Transmission of CWD occurs by direct contact with infected individuals and via contaminated environments. We investigate the seasonal effects of grouping patterns and landscape heterogeneity on direct, pair-wise contacts within and between sex-specific (same or mixed-sex) groups of mule deer (*Odocoileus hemionus*) in central eastern Alberta. First, we establish criteria based on spatial-temporal movements of collared deer to define sex-specific group membership. Second, we model the relative risk of sex and group-specific contacts occurring in a locale based on landscape characteristics. Third, we relate seasonal predictions of the spatial contact probabilities to the risk of deer being CWD-infected in an area based on hunter-harvest, CWD surveillance data. We determined that in winter contacts occurred in areas with higher use by deer, whereas in summer, contact locations were less constrained and were more varied between sexes. Relative contact probabilities of within and between-group male dyads in winter and between-group female dyads in summer were the best predictors of CWD risk in east-central Alberta. Our results relate habitat specific, social behaviours between conspecific mule deer to potential routes of CWD transmission and contribute to CWD research that guides management strategies for an emergent wildlife disease.

## Pathogenesis and Strains

8:40 - 10:00am Friday, 2nd June, 2023

Plaza Ballroom

Moderator Dr. Glenn Telling

8:40 - 9:00am

CWD strain changes following different routes of prion exposure

[Joseph DeFranco](#), Sehun Kim, Jifeng Bian, Jenna Crowell, Glenn Telling

Colorado State University, Fort Collins, USA

### Abstract

Chronic wasting disease (CWD) is a rapidly spreading, uncontrolled prion disease in wild and captive cervids in North America, Europe, and Asia. This pathogen transmits through bodily fluids, shedding skin during infection, is invariably fatal, and has incomparable, robust environmental persistence. Although it is assumed that the primary mode of natural CWD is likely horizontal transmission, either through direct contraction between cervids or indirect infection via contaminated fomites in the environment, there is still limited understanding of how these different transmission routes affect disease pathogenesis. Despite PrP<sup>Sc</sup> (i.e., the pathogenic form of host-encoded PrP<sup>C</sup>) lacking informational nucleic acids, prions share strain diversity analogous to conventional pathogens. These strain properties affect prion infectivity and pathogenesis, PrP<sup>Sc</sup> biochemical properties, and host-range dynamics. It is well established that different routes of prion exposure engender varying disease incubation times; however, this was attributed to the duration of time needed for the pathogen to enter the central nervous system to cause disease. Using newly developed gene-targeted mice models, we

have found that the route of inoculation has a profound effect on prion pathogenesis. Specifically, we have discovered distinct biochemical conformations of the protein, varied titers of the infectious pathogen, and different depositions of prions in the brain. This data suggests that unique prion strains are propagated as a result of the route of prion exposure. This research is critical to understanding the natural transmission and zoonotic potential of CWD, as well as fostering the study of prion biology.

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9:00 - 9:20am

## Evidence for pre existing substrain diversity in a biologically cloned prion strain

Jason Bartz, Tess Gunnels, Taylor York, Benjamin Steadman, Alyssa Block, Ronald Shikiya

Creighton University, Omaha, USA

### Abstract

Prion diseases are a group of inevitably fatal neurodegenerative disorders affecting numerous mammalian species, including humans. Prions are comprised of PrP<sup>Sc</sup>, the prion disease specific conformation of the host encoded prion protein. Prion strains are operationally defined as a heritable phenotype of disease under controlled transmission conditions. Treatment of rodents with anti-prion drugs results in the emergence of drug-resistant prion strains and suggest that prion strains are comprised of a dominant strain and substrains. While much experimental evidence is consistent with this hypothesis, direct observation of substrains has not been documented. Here we show that replication of the dominant strain is required for suppression of a substrain. Based on this observation we reasoned that selective reduction of the dominant strain may allow for emergence of substrains. Using a combination of biochemical methods to selectively reduce drowsy (DY) PrP<sup>Sc</sup> from biologically cloned DY TME-infected brain resulted in the emergence of strains with different properties than DY TME. The selection methods did not occur during prion formation, suggesting the substrains identified preexisted in the DY TME-infected brain. We show that DY TME is biologically stable even under conditions of serial passage at high titer that can lead to strain breakdown. Substrains therefore can exist under conditions where the dominant strain does not allow for substrain emergence suggesting that substrains are a common feature of prions. This observation has mechanistic implications for prion strain evolution and emergence.

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9:20 - 9:40am

## Genotype-by-Sequencing Reveals Population Structure and Fine Scale Spatial Differences in Chronic Wasting Disease Prevalence in Rocky Mountain Elk (*Cervus canadensis*)

Kaneesha Hemmerling<sup>1</sup>, Nathan Galloway<sup>2</sup>, Glen Sargeant<sup>3</sup>, Margaret Wild<sup>1</sup>, Maria Herndon<sup>1</sup>, Sally Madsen-Bouterse<sup>1</sup>, Michelle Mouse<sup>4,1</sup>

<sup>1</sup>Washington State University, Pullman, USA. <sup>2</sup>National Park Service, Fort Collins, USA. <sup>3</sup>U. S. Geological Survey, Jamestown, USA.

<sup>4</sup>U.S. Department of Agriculture, Agriculture Research Service, Pullman, USA

### Abstract

Geographic isolation, disease, and human activity may influence genetic differentiation in wildlife populations. Chronic wasting disease (CWD), a transmissible prion disease, may exert substantial selective pressure in deer (*Odocoileus* spp.) and elk (*Cervus canadensis*). The aims of this study were to develop genotype-by-sequencing (GBS) methodology, quantify population genomics, and discern effects of CWD on genomes of Rocky Mountain elk (N=409) from Wind Cave National Park (WICA), South Dakota, USA. ADMIXTURE analysis revealed two ancestor populations likely founded the current population, aligning with the documented history of WICA elk translocations. Although pairwise differentiation (*F*<sub>st</sub>) showed little difference in genetic structure between three WICA subpopulations (*F*<sub>st</sub> ≈ 0.003), differences were evident when the first two principal components were plotted. Observed heterozygosity in the total population was 0.293. Overlaying PRNP genotypes of amino acid codon 132 and CWD status on the principal component plot supported disease pressure as a contributor to genomic selection. In the three sub-populations present within the park (A, B, and C) observed allele frequencies of lysine at amino acid 132 were 13.5, 19.8, and 30.0% and this corresponded to CWD prevalence of 0.03, 0.21, and 0.29 in subpopulations A, B, and C, respectively. Three loci within 150,000 bases but more than 50,000 bases from PRNP also had different (*p* < 0.038) genotypic and allele frequencies among subpopulations, indicating loci outside of linkage disequilibrium

with the prion locus are under selection in these populations. Monitoring genomics of large wild ungulates with GBS methods may be a more economical management option than the high cost of radio collaring animals and provide information on the presence of disease pressure and landscape division.

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9:40 - 10:00am

## **Distribution of the misfolded isoform of the prion protein in the brain, spinal cord, and peripheral tissues in extremely early to terminal cases of naturally occurring cases of Chronic Wasting Disease in Rocky Mountain elk (*Cervus elaphus nelsoni*)**

Terry Spraker<sup>1</sup>, Tom Gidlewski<sup>2</sup>, Jenny Powers<sup>3</sup>, Tracy Nicholls<sup>4</sup>, Margaret Wild<sup>5</sup>

<sup>1</sup>Colorado State University, Fort Collins, USA. <sup>2</sup>Retired, Fort Collins, CO, USA. <sup>3</sup>National Park Service, Fort Collins, CO, USA. <sup>4</sup>USDA, Fort Collins, CO, USA. <sup>5</sup>Washington State University, Pullman, WA, USA

### **Abstract**

Chronic wasting disease (CWD) is an infectious transmissible spongiform encephalopathy (TSE) in cervids. CWD has been documented in mule deer, elk, white-tailed deer, and moose in North America, reindeer and moose in Norway and Finland, and captive elk in South Korea. The distribution of the misfolded isoform of the prion protein (PrP<sup>CWD</sup>) has been described in the brain and peripheral tissues in mule deer but not in elk. We investigated the spread and distribution of the PrPCWD in the brain and peripheral tissues in 35 adult Rocky Mountain elk with naturally occurring CWD from extremely early to terminal cases. These 35 elk were necropsied, the brain, spinal cord and 100-110 non neural tissues were fixed in 10% formalin, processed for histology, stained with hematoxylin/eosin (H&E) and immuno-stained (IHC) with the anti-prion protein monoclonal antibody F99/97.6.1 (mAb 99) on the Ventana platform. The obex, retropharyngeal lymph node and palatine tonsils were examined in these elk to confirm if the elk were positive or non-detected for CWD. Next an obex score was determined for the CWD positive elk that ranged from 0 (only lymphoid staining with no brain staining) to 10 (terminal cases with extensive lymphoid and brain staining). The obex score delineates a progression of PrPCWD distribution in a single section of brain stem at the level of the obex using the IHC stain and spongiform degeneration using H&E stain from early to terminal stages of CWD. Elk were placed in 11 categories (0 to 10) according to the previous described obex scores. This presentation will demonstrate the progressive spread of PrPCWD in peripheral and neural tissues correlated with the obex scores that will give novel insight into the trafficking of PrPCWD in elk. PrPCWD appeared to spread via the autonomic nervous system throughout the body affecting organs innervated by autonomic nerves. The prion appeared to spread slowly in the brain through the first obex scores (0 to 7) then from 8 to 10 prion spread rapidly and with more intensity. Spongiform encephalopathy colocalized and follows PrPCWD deposition throughout all regions of the brain.

### **Break**

10:00 - 10:20am Friday, 2nd June, 2023  
Plaza Exhibit Foyer

# Pathogenesis and Strains

10:20 - 11:20am Friday, 2nd June, 2023

Plaza Ballroom

Moderator Dr. Glenn Telling

10:20 - 10:40am

## Longitudinal detection of prion shedding in nasal secretions of CWD infected white-tailed deer

Caitlyn Kraft, Nathaniel Denkers, Candace Mathiason, Edward Hoover

Colorado State University, Fort Collins, USA

### Abstract

Chronic wasting disease (CWD) is a fatal neurodegenerative prion disease affecting cervids in North America, Europe and Asia. As CWD continues to spread geographically, the development of minimally invasive detection methods in live animals will be of utmost importance. In previous studies, prions have been shown to replicate in the nasal mucosa, and can be shed in nasal secretions. Less understood is the temporal shedding profile of CWD in nasal secretions throughout disease course. To assess the presence of CWD in nasal secretions over time we collected nasal swabs at 3 month intervals from low dose orally-inoculated white-tailed deer (n=18). Nasal swabs were assayed for CWD prion seeding activity (CWD prions) using iron oxide magnetic extraction combined with real-time quaking induced conversion (IOME RT-QuIC). We correlated IOME RT-QuIC nasal swab results with deer CWD status (tonsil biopsy positivity) and *prnp* genotype. We report the presence of CWD prions in the nasal cavity of 10 of 18 deer (55.5%), 3 to 16 months after detection of tonsil biopsy positivity. Detectable CWD shedding persisted in nine of ten animals (90%). No presence of CWD was detected in nasal swabs collected from eight deer (0%), though all were determined to be CWD positive by tonsil biopsy and QuIC on terminal tissues. Nasal shedding was more common in deer that were in early to mid clinical stages of disease, and in those homozygous for glycine at codon 96. These results suggest nasal swab sampling can be used as a minimally invasive antemortem diagnostic, with higher sensitivity in clinical symptomatic deer.

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10:40 - 11:00am

## Nasal bots: emerging vectors of natural chronic wasting disease transmission

Paulina Soto<sup>1</sup>, Francisca Bravo-Risi<sup>2</sup>, Carlos Kramm<sup>1</sup>, Nazaret Gamez<sup>1</sup>, Rebeca Benavente<sup>1</sup>, J. Hunter Reed<sup>3</sup>, Mitch Lockwood<sup>3</sup>, Denise Bonilla<sup>4</sup>, Tracy Nichols<sup>4</sup>, Rodrigo Morales<sup>1</sup>

<sup>1</sup>UTHealth Houston, Houston, USA. <sup>2</sup>UTHealth Houston, Houston, USA. <sup>3</sup>Texas Parks and Wildlife Department (TPWD), Kerville, USA.

<sup>4</sup>United States Department of Agriculture (USDA), Fort Collins, USA

### Abstract

Chronic wasting disease (CWD) is a fatal neurodegenerative disease affecting farmed and free-ranging cervids. CWD transmission has been shown to occur directly through animal contact and indirectly through contact with contaminated environments. Parasites are known vectors of multiple diseases in animals, including deer; however, the role of parasites in CWD transmission remains unclear. The main objective of this study was to determine the potential of nasal bots (*Cephenemyia* spp.), a common deer parasite, as a CWD vector. Nasal bots were collected from the nasal cavity of non-clinical, naturally CWD-infected white-tailed deer. Nasal bots were also collected from white-tailed deer that tested not-detected for CWD. The CWD seeding activity of the nasal bots was interrogated by PMCA, and disease transmission was evaluated by mouse bioassays (intracerebral injections in Tg1536 mice). To further mimic environmental transmission, nasal bot homogenates were mixed with soil, and plants were grown using this contaminated soil. Both plants and soil were tested for prion seeding activity. PMCA analyses demonstrated CWD seeding activity in nasal bots from captive white-tailed deer. Moreover, CWD-contaminated bots efficiently infected transgenic mice with attack rates and incubation periods suggesting high infectivity. Biochemical characterization of protease-resistant prions and immunohistochemistry confirmed prion infection in experimental mice. Interestingly, plants grown in soil containing CWD-contaminated nasal bots also induced seeding activity



in PMCA reactions. Considering this information, we propose that deer nasal bots could act as relevant vectors of CWD transmission in wild and captive settings.

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11:00 - 11:20am

## **Shedding of Chronic Wasting Disease Prions in Multiple Excreta Throughout Disease Course in White-tailed Deer**

Nathaniel Denkers, Erin McNulty, Caitlyn Kraft, Amy Nalls, Joseph Westrich, Candace Mathiason, Edward Hoover

Prion Research Center, College of Veterinary Medicine and Biological Sciences, Department of Microbiology, Immunology, and Pathology; Colorado State University, Fort Collins, USA

### **Abstract**

Chronic wasting disease (CWD) has infected cervids in South Korea, North America, and Scandinavia, demonstrating efficient transmission, possibly from prions shed in excreta during long course infections. The purpose of this study was to define the longitudinal shedding profile of prions in urine, saliva, and feces in white-tailed deer. Deer were inoculated with CWD, then urine, saliva, and feces were collected every 3-month post-inoculation (MPI) throughout the study. Cohorts were established by PRNP genotype: codon 96GG (n=6) & alternate codons 96GS/103NT (n=6). Urine and saliva were analyzed using iron-oxide magnetic extraction (IOME) and real-time quaking induced conversion (RT-QuIC)(IQ). Feces were subjected to IOME, followed by protein misfolding cyclic amplification (PMCA), then RT-QuIC (IPQ). Within the 96GG cohort, seeding activity was detected in feces from all deer (100%), saliva from 5 of 6 (83%), and urine from 4 of 6 (66%). Shedding occurred at, or just after, the first positive tonsil biopsy. In the 96GS/103NT cohort, seeding activity was detected in feces from 3 of 6 (50%) deer, saliva from 2 of 6 (33%), and urine from 1 of 6 (16%). Shedding was detected >5 months after the first positive tonsil biopsy. IPQ significantly augmented detection in feces by eliminating non-specific background. Negative control samples remained negative. In conclusion: (a) CWD prion excretion occurs throughout infection; (2) PRNP genotype (GG>>GS/NT) influences excreta shedding; and (3) detection sensitivity in excreta can vary with different protocols. These results provide a more complete perspective of prion shedding in deer during CWD infection.

## **Ed Hoover Student oral and poster presentation awards and travel grants**

11:20 - 11:40am Friday, 2nd June, 2023

Plaza Ballroom

-Ed Hoover Student Oral Presentation Award

The Dr. Ed Hoover student oral presentation award was developed to commemorate and celebrate his dedication to mentoring students and his many contributions towards CWD research.

A competition for best oral presentation is open to all students with an accepted abstract. Enrollment in the competition is automatic for those presenters who identified themselves as students on their registration.

A winner and honorable mention for Ed Hoover student oral presentations will be awarded based on a number of set criteria. For more information please see:

-Ed Hoover Student Poster Presentation Award

A competition for best poster presentation is open to all students with an accepted abstract. Enrollment in the competition is automatic for those presenters who identified themselves as students on their registration.

A winner and honorable mention for best student poster presentations will be awarded based on a number of set criteria.

For more information please see: <https://int-cwd-sympo.org/poster-presentation-awards/>

## Closing Remarks

11:40am - 12:00pm Friday, 2nd June, 2023  
Plaza Ballroom



**Bill Werkheiser**, USGS, Science Advisor to the Department of the Interior



**Jason Suckow**, Director, USDA APHIS Wildlife Services National Wildlife Research Center

## Rocky Mountain Arsenal National Wildlife Refuge Tour

1:30 - 5:00pm Friday, 2nd June, 2023

Pre-Registration Required

The tour will include a presentation highlighting the interesting history of this urban Refuge and the ongoing conservation efforts, a drive on the Refuge auto tour route, and up-close viewing the federally endangered black-footed ferret!! Visitors will get the opportunity to see some of the Refuge's bison herd of about 200 and on the tour hear about the bison's role in the Department of Interior Bison Conservation Initiative and contribution to establishing Tribal bison herds.

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