



U.S. Senate Testimony

Committee on Environment and Public Works

12/4/2019

Thank you, Chairman Barrasso, Ranking Member Carper, and members of the committee for the opportunity to discuss with you today a very serious issue facing our nation, Chronic Wasting Disease.

As the Commissioner of the West Virginia Department of Agriculture (WVDA), I believe the establishment of a CWD Task Force is a measure that is long overdue. I will note, I am one of 12 statewide elected Commissioners of Agriculture and as such, I report directly to the citizens of my state. There is a lot of concern surrounding the CWD, especially from our many sportsmen, as well as our captive cervid farmers. Because I am elected, I have the unique opportunity to help shape legislation and policy surrounding issues like CWD. What my staff and I have found is that many of our colleagues from other states operate under laws and regulations much different than our own.

I would also like to point out to the members of this committee that every state Department of Agriculture has different responsibilities. In West Virginia, deer farms are regulated by my Department (WVDA). Deer farms in WV raise whitetail deer and elk, plus exotic reindeer and fallow deer. Wild whitetail deer and elk populations are regulated by the West Virginia Department of Natural Resources (DNR) under the Commerce Department and the Governor. Deer farming regulations originated within the DNR, and authority over deer farms was moved by legislation passed in 2015 to the WVDA. While the WVDA and DNR work closely together on many projects, we sometimes disagree on legislation and rules; the uncertainty of CWD data does not help.

There are various groups and agencies performing some form of research to better understand this slow spreading yet potentially devastating disease. In February of 2019, I asked USDA's Animal and Plant Health Inspection Services (APHIS) to undertake more research concerning Chronic Wasting Disease (CWD). I was concerned there was pressure being placed on legislators to make and enforce laws that were not based on science but a feeling that something must be done. My position has always been that we must legislate from the application of sound science. Farmers are already subject to uncertain weather and uncertain market conditions. Successful farm businesses must have certainty in regulations. This includes the production and interstate transportation of agricultural products, which does include deer, venison, deer embryos, and deer urine, etc.

APHIS responded with an initial literature review which I have attached to this testimony. It is clear from the current state of research we do not have certainty in the science of CWD and the ability to make sound judgments that may affect the livelihood of many farmers and hunting related businesses. I am encouraged that research is ongoing, but more is needed, and it does need to be better coordinated to maximize limited research dollars.

Currently, West Virginia has 36 deer farms up from when I took office in 2017. Deer farming is a growing industry in West Virginia. Deer farming has been growing nationally with the sale of deer urine alone reaching \$16 million in 2016. Arby's restaurants have been testing sales of venison in their stores. For West Virginia, deer farming is an opportunity to diversify our economy and potentially use land that is no longer viable under modern agricultural practices, including our beautiful forests and rolling fields, or even restore value to abandoned mine lands.

To date in West Virginia we have never had a farmed deer test positive for CWD, and we test all animals going to slaughter for meat and 10% of animals harvested in hunting operations. Even though researchers have not been able to replicate the only documented case of the transmission of CWD to a non-human primate via contaminated meat, the Center for Disease Control recommends not eating deer meat from known CWD-positive deer, sick animals, or brain/nervous/lymph tissue from an animal.

West Virginia has long supported the cooperative CWD Voluntary Herd Certification Program between WV deer farms, state animal health and wildlife officials, and APHIS-Veterinary Services. Currently, 30 of 36 WVDA licensed deer farms participate with 28 certified by complying with program standards. Two newer deer farms do not have five years of negative

results at this time. Six licensed hunting preserves are not required to participate as they do not relocate deer. Program goals are to reduce the spread of the disease and decrease risk to farmed deer, improve confidence for trade of farmed deer and deer products, and reduce the risk of transmission and environmental contamination from CWD herds. Program requirements include animal identification, containment fencing, and CWD testing of deer over 12 months of age that die for any reason. Participating deer farms with no positive CWD test become certified as being low-risk after completing five years of negative CWD testing. Interstate movement of farmed deer depends on program participation, compliance with program requirements, and herd certification status.

It is important to point out that any additional studies on this prion-related disease will add value to prion disease research for known human diseases and other domesticated livestock. Deer farms should be a part of this research especially if we as a nation want to develop immunotherapies for domesticated herd protection and eventual suppression and eradication of the disease in the wild.

My opinion is that the spread of CWD is mostly carried out by scavengers. An infected deer that dies by any cause, the disease itself or even being hit by a car, left unattended will soon be consumed by a scavenger most commonly a winged variety, crow or vulture. The viable prion living in the lymphatic system or nervous tissue is then consumed and the prion passes through the digestive tract and eventually deposited miles away. This could explain why the disease has appeared in very tightly closed and monitored herds, or in areas not adjacent to a known infected area.

Therefore, I believe a closed study of infected carcasses and scavengers would yield tremendous insight. Does the prion survive the digestive tract of a predator? We know the prion survives on vegetation outside an animal for a long time. The feces, blood or urine of the host animal may not contain the prion as the animal's natural filter systems block it, so researchers are looking for markers related to prions. It may be awhile before we are confident with a live test for the disease. We may be taking baby steps in slowing the spread, but it must be based on sound science.

In the meantime, as the research is conducted, we can use potentially simple techniques to slow the spread. Akin to what we have done with the "Slow the Spread" program for Gypsy Moth. This takes a properly funded education and communications program. There is simply too much speculation and non-science interpretation of research affecting the business of disease control. This hurts agriculture and sporting businesses.

An example of this are actions I have taken in West Virginia. As I became more convinced scavengers were a primary vector for the spread of the disease, I strongly suggested to the WV Deer Farmers Association at their annual meeting they insist all members make sure all deer carcasses are buried too deep for scavengers to reach. I then made a request to the Department of Highways that they make a more concerted effort to quickly retrieve and bury the road kills within the DNR declared containment area.

In reviewing the proposed legislation, I have three recommendations for improvement:

- 1) At least one of the non-governmental positions on the task force be an experienced and respected deer farm owner/manager
- 2) That the bill clarifies the funding and need for a coordinated education and information campaign between the state agencies, agriculture groups, and the hunting public
- 3) That recommended legislation, if any, resulting from this task force be specific and not left up to interpretation

Thank you once again for this opportunity. I will gladly answer any questions the committee may have.

Kent Leonhardt is a longtime farmer who began his passion for the agriculture at a very young age and fostered that passion into a business operation while still serving in the United States Marine Corps. Towards the end of his military career, Kent bought a farm near Blacksville, WV that had sat abandoned for over 40 years. The farm, where he still lives today, was purchased in 1982, and started cultivating crops and raising livestock in 1997. For twenty years, Kent, with the help of his wife Shirley, raised sheep, cattle and goats and sold hay when there was a surplus available.

Kent received his formal education from University of Missouri, earning a bachelor's degree in Wildlife Management. During his studies, he took a variety of courses covering issues pertinent to agriculture as well as natural resources and environmental protection. Kent furthered his education by earning a Master's in Business Management from Central Michigan University while still on active duty.

After twenty years in the United States Marine Corps, Kent retired from the military in 1996 at the rank of Lieutenant Colonel. During that time, he served on multiple joint service assignments leading men and women during war and peace. Throughout his service, Kent received a variety of decorations including: Legion of Merit, Combat Action Ribbon and eight other personal decorations.

In 2014, Kent was elected to the West Virginia State Senate to serve the people of the Second Senatorial District. The district, at the time, was one of the largest and most rural in West Virginia. It contained parts of or all of the following counties: Marshall, Wetzel, Gilmer, Marion, Monongalia, Tyler, Doddridge, Calhoun, and Ritchie. In 2017, he resigned from his position in the WV State Senate after being elected as the West Virginia Commissioner of Agriculture. He has held that position from 2017.

Together, Kent and Shirley have three sons and six grandchildren. Kent is a member of the Monongalia County Farm Bureau.

Biography for West Virginia Commissioner of Agriculture Kent A. Leonhardt

Current Position: Jan 2017 to present

WV State Senator District 2 - 2015 to 2017

1976 to 1996 United States Marine Corps, Lieutenant Colonel, Retired

Company Commander, Fort Meade, Maryland

Analyst and Watch Officer, NSA Fort Meade

Deputy G-2 (Intelligence), 7th Marine Expeditionary Brigade

Desert Shield/Desert Storm 1990-1991, Led a 68 Marines intelligence unit

Executive Officer 2nd Radio Battalion, Camp Lejeune, NC

USMC Liaison Officer, National Security Agency

Special Intelligence Collection Manager, US Central Command

1996 to present a WV Farmer - Wife and Commissioner restored and grew an abandoned WV Farm.

Education including Military Schools:

*Bachelor Degree in Wildlife Management, University of Missouri 1976

*Masters of Art Business Management Central Michigan University by Extension 1983 while on Active duty

*The Basic School USMC 1976

*Communications Officer School 1977

*Defense Language Institute - Arabic, Monterey, Ca – 1981

*Defense Intelligence College, Bowling AFB - 1987

*USMC Command and Staff College, Quantico, VA - 1990

*Fellow to Director of the National Security Agency, one-year program, 1992-1993

Married to Shirley Leonhardt for 35 years, combined they have 3 sons and 6 grandchildren.

Prion Remains Infectious after Passage through Digestive System of American Crows (*Corvus brachyrhynchos*)

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Abstract

Avian scavengers, such as American crows (*Corvus brachyrhynchos*), have potential to translocate infectious agents (prions) of transmissible spongiform encephalopathy (TSE) diseases including chronic wasting disease, scrapie, and bovine spongiform encephalopathy. We inoculated mice with fecal extracts obtained from 20 American crows that were force-fed material infected with RML-strain scrapie prions. These mice all evinced severe neurological dysfunction 196–231 d postinoculation (\bar{x} = 198; 95% CI: 210–216) and tested positive for prion disease. Our results suggest a large proportion of crows that consume prion-positive tissue are capable of passing infectious prions in their feces (\hat{p} = 1.0; 95% CI: 0.8–1.0). Therefore, this common, migratory North American scavenger could play a role in the geographic spread of TSE diseases.

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Introduction

Transmissible spongiform encephalopathies (TSE) are most likely caused by pathogenic isoforms (PrP^{Res}) of prion proteins [1] that naturally occur across many classes of animals, including mammals and birds [2]. A number of livestock and wildlife species in North America are susceptible to TSE diseases. Mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), elk (*Cervus elaphus*), and moose (*Alces alces*) are susceptible to chronic wasting disease (CWD); domestic sheep and goats are susceptible to scrapie; and domestic cattle are susceptible to bovine spongiform encephalopathy (BSE) (although this disease is rare in North America [3]). These TSE diseases are always fatal to infected animals, and upon death, carcasses allowed to remain in the environment can be scavenged by an array of avian and mammalian scavengers [4].

Mechanisms for the spread of TSE in wild and domestic ungulates are incompletely understood. We hypothesized that avian scavengers have potential to translocate PrP^{Res} in their feces. American crows (*Corvus brachyrhynchos*) are significant avian scavengers of deer carcasses [4], they are migratory, and their overall range [5] includes most areas where TSE diseases occur in North America [6]. Crows forage in groups, traveling up to 80 km/d from communal roosts [5]. Thus, crows have opportunity to encounter PrP^{Res}-infected carcasses, consume infected tissue, and move long distances before depositing feces. Once in the soil, PrP^{Res} may persist >2 years [7,8], potentially enabling increased site contamination over time. For example, residual

contamination of soil with PrP^{Res} caused recurrence of CWD in confined mule deer in Colorado [7] and lateral transmission via environmental contamination is likely an important route of infection [9].

Insects [10,11], poultry [12], and scavengers, including crows [4], have been suggested as passive carriers or dispersers of infectious prions. We found no studies that evaluated passage of PrP^{Res} through avian digestive systems, though several studies have evaluated resistance of PrP^{Res} to mammalian digestive fluids. Ruminant digestive fluids used during in-vitro trials have shown substantial [13,14] to no reduction [15] in Western blot signal after incubation periods of approximately 13–24 h. Shorter incubation times (15–210 min) resulted in intermediate levels of Western blot signal loss [16]. Studies that investigated effects on PrP^{Res} from full passage through rodent digestive systems found scrapie and BSE PrP^{Res} present in mouse feces [17] and scrapie PrP^{Res} in hamster feces (ca. 5% of original dose excreted 24 h postinoculation) [18]. Thus, it appears that mammalian digestive fluids and processes can reduce PrP^{Res} concentration but are unlikely to eliminate it.

Proteolysis occurs in the avian digestive system due to the presence of hydrochloric acid (HCl) and the proteolytic enzymes pepsin, trypsin, chymotrypsin and various peptidases [19,20]. Although experimentally induced hypoacidity was associated with reduced scrapie infection rates in mice [21], it is unlikely that gastric HCl would fully degrade PrP^{Res} in the crow digestive system given extreme temperature and concentration required

[22] and mild conditions present in the avian gut [19,23]. Although early investigations suggested that trypsin reduced scrapie titer under certain circumstances [1,24], subsequent studies found pepsin and trypsin were not effective for reducing infectivity of scrapie and BSE PrP^{Res} [25] or variant Creutzfeldt-Jakob disease PrP^{Res} [26]. Thus, there is little evidence to suggest that the crow digestive system would eliminate PrP^{Res} infectivity prior to excretion of feces. Similar arguments can be made for nonruminant mammals because of similarities in endogenous enzymes in vertebrate digestive systems [27], yet PrP^{Res} was substantially reduced by passage through hamster digestive systems [18].

Little is known about effects of avian digestive systems on infectivity of PrP^{Res}. As a first step in understanding the potential role of avian scavengers in TSE transmission, we tested the hypothesis that readily available mouse-adapted scrapie PrP^{Res} can remain infectious after passage through the digestive tract of crows. Results of our study support this hypothesis.

Materials and Methods

We evaluated infectivity of the RML Chandler strain (RML) of mouse-adapted scrapie [28] (obtained from Rocky Mountain Laboratories, Hamilton, MT) after passage through digestive systems of crows. Crows were captured during winter in central Oklahoma, USA. We used mouse-brain source material from uninfected (normal) and terminally ill RML-infected C57BL/6 mice (Hilltop Lab Animals, Scottsdale, PA; this strain used throughout study). We separately pooled and homogenized infected and normal mouse brains and diluted portions of each homogenate 1:10 w/v in sterile phosphate-buffered saline (SPBS). We estimated passage time through the alimentary canal by gavaging 1 crow (not part of the experimental group) with 5 ml of whole egg mixed with blue dye; by 4 h postgavage all stained feces had been excreted. We withdrew feed (but not water) from study crows approximately 17 h pre-gavage. We randomly allocated 25 crows to treatment groups and gavaged each crow with 5 ml of either PrP^{Res}-infected (n = 20) or normal (n = 5) mouse-brain homogenate diluted 1:10 w/v in SPBS (Table 1). We then transferred each crow to an individual single-use cage. At 4 h postgavage, we collected and pooled all feces within each cage. We homogenized crow-specific pooled feces and gamma irradiated them at 24,000 Gy to destroy viruses and microbes. For each crow, we then diluted a 500 µl sample of fecal homogenate in SPBS to a total volume of 10 ml, centrifuged it for 15 min at 13,730 m/s², and extracted the supernatant for use as inoculum for mice. We removed solids to minimize risk of toxicity to mice from uric acid contained in bird feces. Crows were not held or examined after collection of fecal samples.

We randomly allocated 5 mice/crow to treatment groups (Table 1). Mice received crow-specific fecal supernatant from PrP^{Res} or control crows (CF+ and CF− groups, respectively), or PrP^{Res}-infected or normal mouse brain homogenate diluted to 1:100 w/v in SPBS (MB+ and MB− groups, respectively). We intraperitoneally inoculated each mouse with 1 ml of either crow fecal supernatant or diluted mouse brain homogenate.

All 5 mice/crow, or 5 mice/MB treatment group, were caged together under biosafety level 2 conditions. We monitored mice daily until all those in PrP^{Res} treatment groups expressed clinical symptoms of mouse scrapie and were thereafter euthanized. Remaining mice were monitored every 2 d until study termination at 365 d postinoculation (dpi). We scored mice for each of 6 clinical symptoms of mouse scrapie (kyphosis, ataxia, stiff tail, lack of grooming, emaciation, and lethargy), where 0 = none visible, 1 = moderate, and 2 = severe. We euthanized mice when total

Table 1. Experimental design used to estimate proportion of crows able to pass infectious RML scrapie prion (PrP^{Res}) in feces (numbers of animals).

Treatment group ^A	Crows	Mice ^B
CF+	20	100
CF−	5	25
MB+	0	10
MB−	0	5

^AMice intraperitoneally inoculated with gamma-irradiated crow fecal (CF) extract from crows gavaged with PrP^{Res} (+) or control (−) mouse brain homogenate; additional control mice were inoculated with mouse-brain homogenate with (MB+) or without (MB−) PrP^{Res}.

^BFive mice were randomly allocated to each crow and housed together in 1 cage postinoculation. Additional control mice were allocated randomly to MB treatment groups and 5 mice/treatment group were housed together in 1 cage postinoculation.

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daily scores reached ≥ 8 for 1 d, ≥ 6 continuously for 3 d, or at 365 dpi. Brains were immediately harvested and stored at -70°C for analysis. Samples from harvested brains (1:10 w/v homogenate) were tested at Colorado State University's Veterinary Diagnostic Laboratory for PrP^{Res} using the ELISA-based Bio-Rad TeSeE BSE rapid assay (Bio-Rad Laboratories, Hercules, CA, USA) to confirm scrapie diagnosis.

We used exact methods [29] to estimate a 95% confidence interval (CI) on the proportion of crows able to excrete infectious prions in feces (SAS PROC FREQ [30]). We used Fisher's exact test, due to low count (i.e., 2) in 1 cell of the 2×2 contingency table, to evaluate whether early death (≤ 3 dpi) was associated with source of CF inoculum (PrP^{Res} or control). We estimated means and 95% CI for incubation time or time-to-death (contingent on surviving > 3 dpi) for CF+ and MB+ mice using general linear mixed modeling [31], where cage was a random effect to account for clustering of mice within cages (SAS PROC GLIMMIX [30]). Traditional time-to-event (or survival) analyses were not required for CF+ and MB+ mice because none were censored > 3 dpi. As most CF− mice were censored at study termination, we tested for equality of survival functions between CF+ and CF− using the log-rank test (SAS PROC LIFETEST [30]).

Ethics Statement

The Institutional Animal Care and Use Committee of the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center approved all procedures used in this study (QA-1406).

Results

All 20 crows gavaged with scrapie-infected mouse brain transmitted PrP^{Res} to mice via fecal inoculum (estimated proportion: $\hat{p} = 1.00$, CI: 0.83–1.00). Sixteen mice from CF+ and 2 from CF− groups died ≤ 3 d postinoculation (likely from residual uric acid toxicity; Table 2). No early deaths occurred in MB groups and estimated probabilities of early death were not statistically different between CF+ ($\hat{p} = 0.16$) and CF− ($\hat{p} = 0.08$) mice (Fisher's exact $P = 0.524$). After these early deaths, 2 crows were represented by only 1 mouse/crow and all other crows were represented by 3–5 mice/crow. Surviving mice appeared healthy until onset of clinical symptoms of mouse scrapie. Based on scoring for multiple clinical symptoms, we euthanized mice in MB+ and

CF+ groups 181–231 dpi (Fig. 1). These mice subsequently tested positive for PrP^{Res} (Table 2). On average, MB+ mice had shorter incubation times (by 15 d) than CF+ mice (Fig. 1). We observed no clinical symptoms in MB– or CF– control mice. All MB– mice lived to study termination at 365 dpi, though 3 CF– mice died at 251–303 dpi. Time to death was significantly longer for CF– than for CF+ mice ($\chi^2_1 = 71.0, p < 0.0001$). One of these CF– mice (251 dpi) tested positive for PrP^{Res}. This unexpectedly positive mouse was inoculated directly after 5 MB+ mice and may have been inadvertently exposed to PrP^{Res}-positive material.

Discussion

We tested the hypothesis that PrP^{Res} would remain infectious after passage through the digestive tract of crows. After inoculation with fecal supernatant from crows gavaged with PrP^{Res}-infected material, we observed clinical disease and obtained positive results from ELISA in all 84 CF+ mice that survived >3 dpi. Thus, we confirmed passage of infectious PrP^{Res} through all 20 crows gavaged with infected material. We conclude that 83–100% of crows from the population we sampled can excrete infectious RML PrP^{Res} in feces under conditions similar to those in our study.

The MB+ mice developed clinical scrapie 15 d earlier than CF+ mice indicating inoculated dose of PrP^{Res} infectivity was likely lower for CF+ mice. We inoculated MB+ and MB– mice to demonstrate that brain source materials were infectious or not infectious, respectively, not to serve as standards for titer assessment. However, comparison with unpublished titration results from intraperitoneal inoculation of RML mouse scrapie into C57BL10 mice (Ann Ward and Sue Priola, Rocky Mountain Laboratories, personal communication) suggest MB+ mice re-

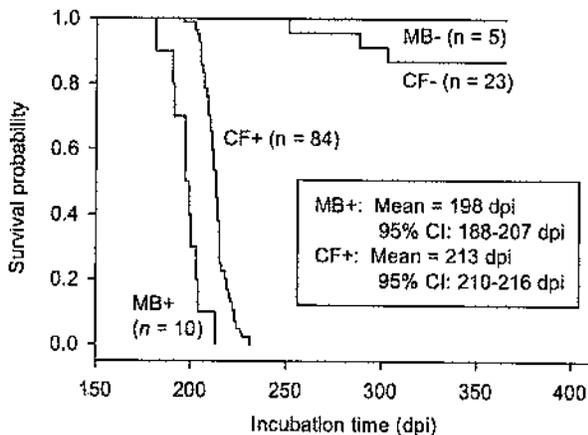


Figure 1. Survival functions for treatment groups of mice. Twenty-five crows were fed infected (PrP^{Res}) or normal (control) mouse brain homogenate. Five mice/crow were subsequently inoculated with crow fecal extract from PrP^{Res} (CF+) or control (CF–) crows. Additional control mice were inoculated with mouse-brain homogenate with or without PrP^{Res} (MB+ and MB–, respectively). Sample sizes reflect early deaths of 16 mice ≤ 3 d postinoculation (dpi). Mean and interval estimates of survival time for MB+ and CF+ groups showed these groups were significantly different, indicating different dose levels of PrP^{Res} in crow fecal extracts compared to mouse brain homogenate. Time to death was significantly longer for CF– than for CF+ mice ($\chi^2_1 = 71.0, p < 0.0001$). Because all mice exposed to CF+ extracts died of transmissible spongiform encephalopathy (given survival >3 dpi), all 20 crows gavaged with PrP^{Res}-infected mouse brain homogenate passed infectious doses of PrP^{Res} to mice via fecal extracts.
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Table 2. Numbers of mice by treatment group that suffered early inoculation-related death, exhibited clinical symptoms of prion disease, and tested positive for scrapie prion (PrP^{Res}) by ELISA^A.

Treatment group ^B	Early death ^C	Clinical disease ^D	PrP ^{Res} detected
CF+	16 (100)	84 (84)	84 (84)
CF–	2 (25)	0 (23)	1 (23)
MB+	0 (10)	10 (10)	9 (9)
MB–	0 (5)	0 (5)	0 (4)

^ANumbers in parentheses indicate sample size.

^BMice intraperitoneally inoculated with gamma-irradiated crow fecal (CF) extract from crows gavaged with PrP^{Res} (+) or control (–) mouse brain homogenate; additional control mice were inoculated with mouse-brain homogenate with (MB+) or without (MB–) PrP^{Res}.

^CMice that died ≤ 3 d postinoculation, presumably from fecal uric acid toxicity. These mice were removed from the data set.

^DMice that achieved a minimum threshold score, based on multiple symptoms such as kyphosis, ataxia, stiff tail, lack of grooming, emaciation, and lethargy, demonstrating strong clinical evidence of prion disease.

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ceived approximately 10-times more infectivity than CF+ mice. Dilutions of brain and fecal material with SPBS (see Methods) indicate that the amount of infectivity inoculated into MB+ mice would have been about double that of CF+ mice, assuming no influence on concentration of infectivity due to passage or centrifuge processing. It is reasonable to expect some loss of infectivity after removing solids from diluted crow feces by centrifugation. It is also possible that some degradation or absorption of infectivity occurred during passage through crow alimentary tracts.

Our study clearly shows that RML PrP^{Res} can persist after passage through the crow alimentary tract. As there is variability in resistance of different strains of PrP^{Res} to degradation [32–36], we cannot definitively state that passage of strains of concern would occur. However, RML PrP^{Res} has been shown more sensitive to degradation than TSE field isolates after 4 h exposure to enzymatic digestion [36]. Therefore, results of our study likely underestimate potential for prion passage through the alimentary canal of crows. Further experimental trials involving TSE prions obtained from ovine, bovine, and cervine carcasses would be required to definitively evaluate passage of natural TSEs through digestive systems of scavengers and predators. Other additional research topics could include in-vitro evaluation of PrP^{Res} degradation in crow digestive fluids; effects of solid, semisolid, and liquid delivery of infective materials on passage rate and residual infectivity in feces; postexcretion continued enzymatic and bacterial degradation of infectivity in feces; infectivity of feces excreted >4 h postgavage; susceptibility of crows to TSE disease and potential for postinfection shedding of PrP^{Res} in feces.

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Author Contributions

Conceived and designed the experiments: KV PN. Performed the experiments: JF JP KV PN. Analyzed the data: GP. Wrote the paper: GP KV.

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Cervids: CWD Voluntary Herd Certification Program



The **CWD Herd Certification Program (HCP)** is a cooperative effort between the Animal and Plant Health Inspection Service (APHIS), State animal health and wildlife agencies, and farmed cervid owners. APHIS coordinates with these State agencies to encourage cervid owners to certify their herds and comply with the CWD Herd Certification Program Standards.

The goal of the HCP is to provide a consistent, national approach to control the incidence of CWD in farmed cervids and prevent the interstate spread of

CWD. Achieving this goal will ultimately result in several important long-term outcomes, including:

- Healthy cervids (both farmed and wild populations) with a reduced risk of CWD. Increased confidence that HCP-certified herds are low risk for CWD infection.
- Strong trade of cervid animals and products (increased market confidence).
- Reduced risk of transmission from, and environmental contamination by, CWD-positive herds.

This goal is accomplished through the establishment of the national CWD herd certification program and interstate movement requirements for CWD-susceptible cervids found in title 9 of the Code of Federal Regulations (CFR) parts 55 and 81 . These regulations are written as performance-based regulations that describe the legally required outcomes.

National CWD HCP requirements for all enrolled herd owners include fencing, individual animal ID's, regular inventories, and testing of all animals over 12 months that die for any reason. With each year of successful surveillance, participating herds will advance in status until reaching five years with no evidence of CWD, at which time herds are certified as being low risk for CWD. Interstate movement of animals from herds will depend on participation in the program, compliance with program requirements, and herd certification status.

Twenty-eight (28) states are currently participating in the program as of December 2017. States interested to join the National CWD HCP must submit an application and supporting documents to APHIS. To find out more about the application process, please see link below.

- List of Approved State CWD Herd Certification Programs (HCP)
- Application Document for States
- VS Form 11-2

The CWD Herd Certification Program Standards

The **CWD Herd Certification Program Standards** provide detailed descriptions of acceptable methods for complying with the legal requirements in 9 CFR parts 55 and 81, which are performance-based regulations that describe the legally required outcomes. The Program Standards also provide guidance for sample collection, biosecurity, and disposal:

Part A, Herd Certification Program, describes acceptable methods to meet the minimum requirements to certify farmed cervid herds for interstate movement.

Part B, Guidance on Response to CWD, describes acceptable methods to meet the minimum requirements to respond to the finding of CWD in farmed cervid herds.

The methods in these Program Standards have been approved by the APHIS Administrator. Alternatively, States may propose other methods/approaches to meet the regulatory requirements. These alternative proposals should be submitted in writing to APHIS for approval. States may also have additional or stricter requirements that exceed the minimum requirements described in the CWD regulations.

The Program Standards will be reviewed regularly by APHIS and, as appropriate, representatives of the cervid industry and State and Federal agencies. A notice will be published in the Federal Register to inform stakeholders of any revisions APHIS plans to the Program Standards.

- CWD Herd Certification Program Standards , May 2019
- CWD Program Standards Review Working Group Summary Document 2016
- Regulatory Sample Collection

Interstate Movement of Cervids

NOTICE: No farmed or captive deer, elk or moose may be moved interstate unless it meets the requirements listed in Part 81 of the Code of Federal Regulations (CFR).

- ADT/Traceability Fact sheet for interstate transport of cervids
- Requirements for Interstate Transport of Wild Caught Cervids
- Surveillance and Testing Requirements for Interstate Transport of Wild Caught Cervids

Related Links

- CWD Rule
- CWD Status of Captive Herds
- Revised Program Standards: Overview of Changes: Presentation
- 2018 Revised CWD Program Standards: What You Need to Know- for Cervid Producers
- 2018 Revised CWD Program Standards: What You Need to Know- for Accredited Veterinarians and Sample Collectors

The Chronic Wasting Disease Transmission in Cervidae Study Act

ISSUE SUMMARY

Chronic Wasting Disease (CWD) is a contagious neurological disease affecting deer, elk, and moose (cervids). Like Bovine Spongiform Encephalopathy, commonly known as “mad cow disease”, CWD is a form of transmissible spongiform encephalopathy (TSE) and is likely transmitted by prions. These prions affect brain function and for some TSEs, are linked to degenerative conditions in humans.

Recent findings from studies regarding transmissibility of CWD to non-cervid species conflict in their conclusions. In 2017, Canadian researchers published findings detailing the first documented transmission of CWD through consumption of contaminated meat in non-human primates. Subsequent investigations were not able to reproduce the results; nevertheless, the Centers for Disease Control [continues to recommend](#) that hunters should “strongly consider having the deer or elk tested for CWD before you eat the meat”, prevent mixing of product during processing, and avoiding consumption of meat from animals that test positive for CWD.

LEGISLATIVE NEED

CWD has been documented to affect both captive and live herds in 26 states and recently was [identified in a 4th Canadian province](#) (see [attached map](#)). In the United States, state fish and wildlife agencies have primacy over wildlife management. Accordingly, state agencies have largely been responsible for conducting research, and for developing and implementing best management practices (BMPs).

Despite the widespread prevalence of CWD, very little is known about common vectors for transmission. BMPs vary significantly among states; the Association for Fish and Wildlife Agencies (AFWA) [recently adopted new BMPs](#) for CWD at their September 2018 AFWA Fish and Wildlife Health Committee Meeting. AFWA identified the need for additional research to identify:

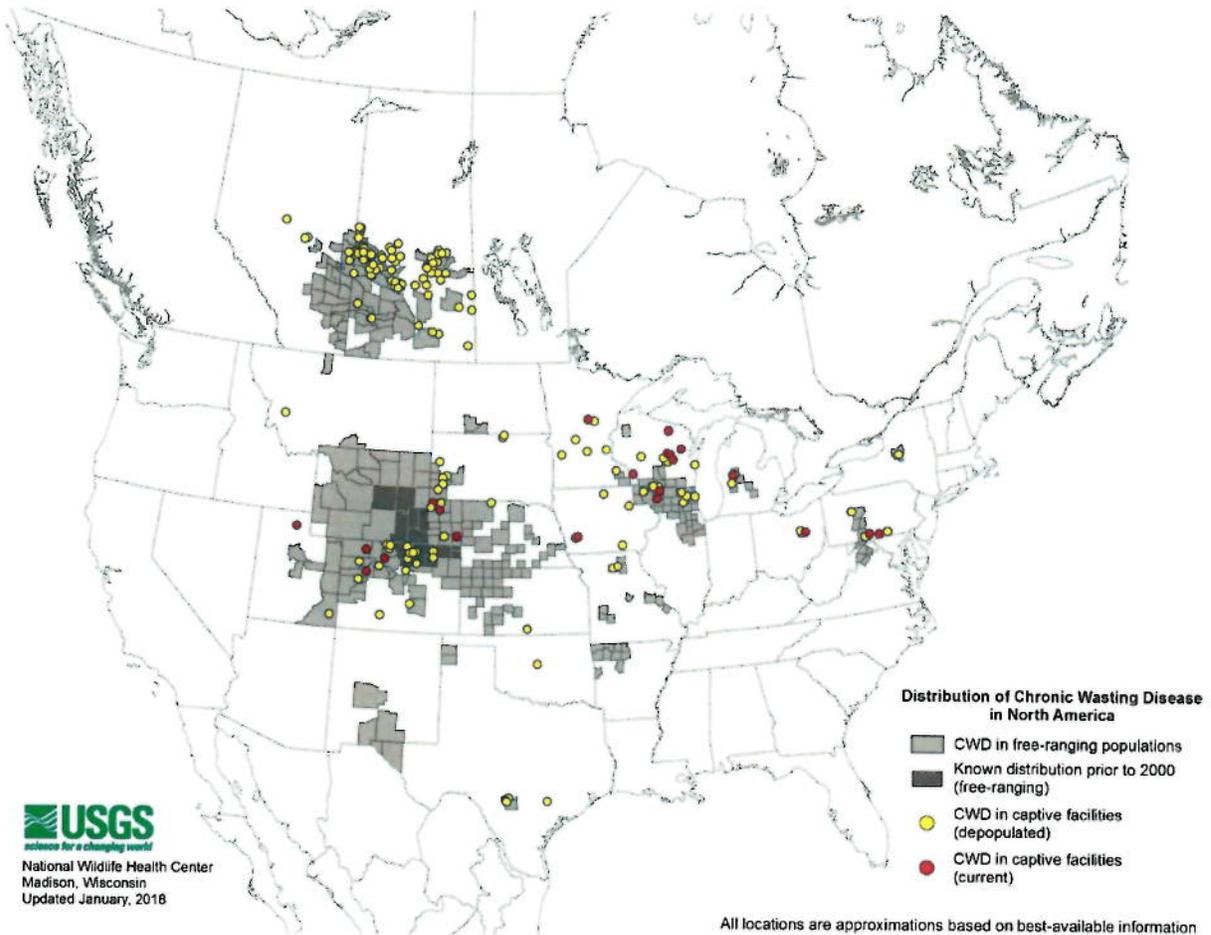
1. The most effective techniques for prevention, surveillance, and management; prion detection and diagnostics; and disease epidemiology.
2. Human dimensions issues such as the impact of CWD on hunting practices and on hunting-related expenditures.
3. The cost of CWD to state and provincial economies.
4. The costs of CWD to wildlife agencies to facilitate budget planning and to landowners, hunters, and other stakeholders.
5. Other sources of funding for CWD prevention, surveillance, and management

The U.S Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) operates a [CWD Herd Certification Program](#) for farmed cervids. Captive herds must participate in the program and be certified to move animals interstate. The program is reactive, rather than proactive. Given the existing risk to wildlife health, economic impact of wildlife loss, and the potential risk to human health, more data is needed. This data can be used to inform a more comprehensive future approach to CWD prevention.

SUMMARY

The Chronic Wasting Disease Transmission in Cervidae Study Act addresses the needs identified by state agencies through AFWA. The bill requires the USDA Secretary to enter into an arrangement with the National Academies of Sciences to review current data and BMPs from the CWD Herd Certification Program and state agencies regarding:

1. Pathways and mechanisms for CWD transmission
2. Areas at risk and geographical patterns of CWD transmission
3. Gaps in current scientific knowledge regarding transmission to prioritize research to address gaps



United States

- [Alabama](#)
- [Arizona](#)
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- [Illinois](#)
- [Iowa](#)
- [Kansas](#)
- [Maryland](#)
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- [Saskatchewan](#)

Gothard, Robin

From: Forbes, John T - APHIS <John.Forbes@aphis.usda.gov>
Sent: Monday, March 04, 2019 4:04 PM
To: Leonhardt, Kent
Cc: Hatton, Joseph
Subject: CWD
Attachments: Nichols Coyote Passage of Prion.pdf; VerCauteren Prion thru Crows.pdf

Commissioner Leonhardt,

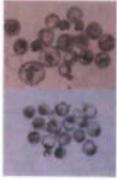
You mentioned during our meeting in January that you were curious about the potential role of predators in spreading CWD so I checked into it and there's already been some research in the area. Attached are two papers – one that studied crows and the other that studied coyotes – which show it is possible to have prions pass through these two species based on their study methodology.

Please let me know if you have any questions or if there's anything we can help you with.

John

John Forbes
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John.Forbes@aphis.usda.gov

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CWD Prions Remain Infectious after Passage Through the Digestive System of Coyotes (*Canis latrans*)

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**CWD Prions Remain Infectious after Passage Through the Digestive System of Coyotes
(*Canis latrans*)**

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Keywords

Chronic Wasting Disease, Coyotes, Environmental Contamination, Feces, Prions, Transmission, Scavengers

Abstract

Chronic wasting disease (CWD) is a geographically expanding prion disease of wild and captive cervids in North America. Disease can be transmitted directly, animal to animal, or indirectly via the environment. CWD contamination can occur residually in the environment via soil, water, and forage following deposition of bodily fluids such as urine, saliva, and feces, or by the decomposition of carcasses. Recent work has indicated that plants may even take up prions into

the stems and leaves. When a carcass or gut pile is present in the environment, a large number of avian and mammalian species visit and consume the carrion. Additionally, predators like coyotes, likely select for disease-compromised cervids. Natural cross-species CWD transmission has not been documented, however, passage of infectious prion material has been observed in the feces of crows. In this study we evaluated the ability of CWD-infected brain material to pass through the gastrointestinal tract of coyotes (*Canis latrans*) following oral ingestion, and be infectious in a cervidized transgenic mouse model. Results from this study indicate that coyotes can pass infectious prions via their feces for at least three days post ingestion, demonstrating that mammalian scavengers could contribute to the translocation and contamination of CWD in the environment.

Introduction

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy, or prion disease, of deer (*Odocoileus virginianus* and *O. hemionus*), elk (*Cervus elaphus*) and moose (*Alces alces*). First identified in 1967 in a wildlife research center in Colorado, CWD is the only prion disease enzootic to wild animals.¹ Transmission of CWD can occur directly, animal to animal², or indirectly through the environment.³ Contamination of the environment can occur by deposition of bodily fluids⁴⁻⁶ or by decay of infected carcasses.³ Ingestion or inhalation of contaminated soil particles can also lead to disease transmission.^{7, 8}

Each year the number of states reporting incidences of CWD in captive or wild cervid populations increases. Currently, 21 states have been affected (<http://www.cwd-info.org/index.php/fuseaction/about.map>). In some regions the spread has been contiguous, such as that seen in Colorado, Wyoming, and Nebraska. Other incidences are far removed from known CWD-positive foci. The mechanisms for this expansion are unclear, and likely vary by circumstance. Several human behaviors, such as movement of captive cervids⁹ and the dumping of CWD-positive carcasses from hunter kills in CWD-negative regions¹⁰, have likely contributed to the expansion, but may not explain all incidences.

The role scavengers play in the spread of the disease has been evaluated primarily from a cross-species transmission aspect.^{11, 12} A wide variety of avian and mammalian scavenger species have been documented to feed upon deer carcasses and gut piles.¹² The array of tissues that contain CWD include brain, eyes, lymph nodes, neural tissue, heart, spleen, and muscle¹³⁻¹⁷, and are all readily accessible in both carcasses and gut piles. Common scavengers from CWD-enzootic areas (raccoons (*Procyon lotor*), opossums (*Didelphis virginiana*) and coyotes (*Canis latrans*)) have been evaluated for the presence of CWD in their tissues, but no evidence of CWD

was detected, suggesting that they do not play a direct role in transmission or become infected.¹¹ They may, however, play a more indirect role. Recent work demonstrated that infectious mouse-adapted scrapie prions could be viably passed in the feces of crows (*Corvus brachyrhynchos*) after ingestion.¹⁰ Deposition of infectious feces from scavengers could then be another unexplored mode for environmental contamination. Mammalian scavengers, such as coyotes, are of particular interest in western states such as Colorado and Wyoming, where there are both a high number of CWD-infected deer and elk, and coyotes.

Coyotes are opportunistic and widespread carnivores found throughout much of North America and everywhere CWD is enzootic in the wild.¹⁸ Their diet is composed primarily of rodents and lagomorphs, however, diet composition can vary seasonally and by geographical location and include ungulates.^{19,20} For example, coyotes in the Black Hills of South Dakota feed preferentially on white-tailed deer (*Odocoileus virginianus*) throughout the year, with the highest consumption occurring (72%) in the winter.²¹ In addition to predation, coyotes will opportunistically forage on carcasses and entrails left by human hunters.²²⁻²⁴ Both predation and scavenging exposes coyotes to CWD in affected regions.

Little is known about the degradation of CWD-infected tissue and infectivity after passage through the gastrointestinal tract of mammalian scavengers. In this study we investigated the potential for coyotes to translocate infectious CWD prions, via feces, after oral consumption of CWD-infected elk brain, utilizing a cervidized transgenic mouse bioassay.

Methods and Materials

Coyotes

All procedures involving animals were approved by the Institutional Animal Care and Use Committee at the USDA National Wildlife Research Center in accordance with the USDA Animal Welfare Act Regulation, CFR, title 9, chapter 1, subchapter A, parts 1-4. Six coyotes, two males and four females, were transported from the National Wildlife Research Center (NWRC) field station in Logan, Utah to the NWRC headquarters in Fort Collins, Colorado. Upon arrival, coyotes were individually housed in outdoor kennels with den boxes for three weeks to allow time for acclimation. During this time, feces were collected from each coyote for pre-exposure controls and frozen at -80°C . Coyotes were given water *ad libitum* and were fed dry dog food once a day. Prior to initiation of the study, coyotes were given a small amount of CWD-negative elk brain homogenate to test for palatability. The coyotes readily ate the homogenate when housed in their outdoor enclosures.

Two coyotes were placed in the control group and remained in their outdoor kennels, while the remaining four were transported to indoor wire runs (Table 1). After DPI 5 fecal collection, coyotes were sedated intramuscularly with ketamine and xylazine, then euthanized intravenously with euthanasia solution. Complete necropsies were conducted, and the brain and lymph nodes placed in 10% buffered formalin.

Inoculum Preparation

CWD-negative and positive 10% elk brain homogenates (wt/vol) were prepared using 1X phosphate buffered saline (PBS) (Invitrogen) in a blender, then aliquoted into 50 ml volumes and stored at -80°C until needed. The CWD-negative elk brain was an archived sample from a captive cull and the CWD-positive elk brain was an archived sample from a terminally ill captive elk. The CWD status of the elk brains was verified by Western blot as previously described.⁸

CWD-negative brain was prepared first in a new blender, then the CWD-positive inoculum was prepared.

Oral Inoculation

After being moved indoors for biosecurity reasons, treatment coyotes were given an acclimation period of two days before the start of the experiment. Dry diet was removed 12 hours prior to introduction of brain homogenate inoculum. A 50 ml aliquot of elk brain homogenate (normal or infected) was thawed for each coyote and placed in a clean bowl and mixed with approximately 1 g of red glitter to help visualize passage time through the alimentary canal. Relocation from outdoor to indoor kennels affected the coyotes' willingness to eat the brain homogenate. The addition of a small amount of diced raw chicken, or in one case, wet fish-flavored cat food was required to get them to eat. The afternoon after ingestion of the brain homogenate, they received dry dog food.

Feces Collection

Feces from all coyotes was collected the morning following elk brain homogenate consumption and at six time points: one day prior to the initiation of the study, and for five consecutive days following inoculum ingestion. After collection, fecal samples were frozen at -80°C .

Feces Protein Misfolding Cyclic Amplification (PMCA)

A 10% fecal homogenate was generated with 200 mg of feces placed into a 1.5 ml tube with 2.5 mm glass beads (BioSpec), and 1 ml of PMCA buffer (150 mM NaCl, 4 mM EDTA, in 1X PBS), then homogenized in a Blue Bullet homogenizer (Next Advance) for 2-4 min. Once homogenized, samples were centrifuged at 12,000 rpm for 20 sec, then 600 μl of supernatant was removed and mixed with 600 μl of the above PMCA buffer with 2% triton-X (Sigma Aldrich) added. Samples were mixed well and shaken on a heat block at 37°C for 20 min at 800 rpm,

then centrifuged for 5 min at 2000 rpm. Supernatant was removed and stored at -80°C . To help develop an appropriate bioassay design, fecal samples were assessed for proteinase K resistance, a marker for infectivity, by PMCA as previously described by Pulford, *et al.*⁵ Amplified samples were visualized by western blot as previously described.²⁵

Coyote Immunohistochemistry

At necropsy, retropharyngeal and mesenteric lymph nodes were preserved in 10% buffered formalin. One week after collection, tissues were placed in plastic cassettes and allowed to fix for an additional two days. Tissue slices 5 μm thick were mounted on positively charged glass slides (Fisher Scientific) for visualization and evaluation.²⁶ Antigen retrieval with formic acid and hydrated autoclaving was performed prior to visualization of PrP^{CWD} , a biomarker for CWD, by staining with F99/97.6.1 antibody. Following antibody, slides were by incubated with alkaline phosphatase-conjugated anti-mouse IgG secondary antibody and visualized using an automated immunostainer and an alkaline phosphatase red kit (Ventana). Slides were counterstained for four minutes with hematoxylin at 37°C . PrP^{CWD} was visualized as brownish granular staining.

TG12 transgenic mice

To test the infectivity of the coyote feces, a transgenic mouse bioassay was conducted in which 77 transgenic cervidized TG12 mice of both sexes, between two and five months of age, were inoculated intracerebrally with coyote feces homogenate at four time points: 1- prior to ingestion of inoculum, 2- one day after ingestion, 3- two days after ingestion, 4- three days after ingestion (Table 2). TG12 transgenic mice were generated as previously described²⁷, and express the elk prion protein at twice the level of mouse prion protein in the FVB background strain.

Feces preparation and inoculation of transgenic mice

A 10% fecal homogenate (wt/vol) was generated in the same fashion as the brain homogenate with the exception of utilizing DI water instead of PMCA buffer, with 100 units/mL penicillin and 100 µg/mL streptomycin (Invitrogen) added. Inoculum was allowed to incubate at room temperature for 30 minutes, then inoculum was sonicated in a 3000MP water bath sonicator (Misonix) for 30 seconds at power 70 prior to intracerebral inoculation. Mice were anesthetized with isoflurane gas until unresponsive to toe pinch. An insulin syringe was employed to intracerebrally inoculate 30 µl of the feces inoculum, 3 mm deep through the coronal suture, 3-5 mm lateral of the sagittal suture.

When mice presented with severe ataxia or reached 405 DPI they were euthanized.

Results

Coyotes

Both control coyotes, and coyote #135 readily ate the brain homogenate. Two others consumed the homogenate after mixing it with a small amount of diced raw chicken and the fourth after mixing the homogenate with a tablespoon of fish-flavored wet cat food.

Treatment coyote #137 did not defecate on days post inoculation (DPI) 2, therefore the data reflects feces from DPI 3 and 4. Red glitter was utilized to give a general idea of the passage time from ingestion to defecation and was observable in feces on DPI 1 and 2.

Protein Misfolding Cyclic Amplification (PMCA)

To ascertain the appropriate number of days of feces collection to test in the transgenic mouse bioassay, PMCA was conducted to amplify minute levels of PK-resistant prions from the coyotes feces from each of the six collection time points. All fecal samples collected prior to ingestion were negative after six rounds of PMCA, as were all of the samples from coyotes in the control group (data not shown). The four coyotes fed CWD-positive brain homogenate had prion

amplification on DPI 1, and only one coyote, #137, had amplification on DPI 2. No signal was detected in DPI3-5 (data not shown). Based on this information, the bioassay was designed to assess feces from DPI 1-3.

Coyote Immunohistochemistry

Immunohistochemistry for protease-resistant prions was conducted on head and mesenteric lymph nodes from the study coyotes to look for residual inoculum, and no evidence of CWD prions was detected in the tissues (Fig 1).

Transgenic mouse bioassay

The transgenic mouse bioassay revealed that feces from coyotes fed infectious brain material could pass infectivity for at least three days after ingestion. Mice were euthanized at the presentation of severe ataxia, and disease was confirmed by western blot (data not shown). Several mice proved to be transgenic knockouts when their genetics were rechecked at the end of the study. As a result, these mice were excluded from results as they do not become sick in the absence of the prion protein. No mice became sick after being inoculated with pre-exposure feces. Half of the four coyotes fed infectious elk brain homogenate passed infectivity on DPI 1, 2 and 3. It is interesting to note that passage of infectivity varied greatly between animals, with one animal passing disease on only DPI 1, while one only began to pass infectivity on DPI 2 and 3, one coyote passed infectivity on all three days, however, this animal did not defecate on DPI 2 and feces collected represents three and four days after ingestion. And finally, one coyote, did not pass infectivity on any of the days (Table 2, Figure 2).

No significant difference was seen in mouse survival times between DPI 1, 2, and 3 (one way ANOVA, $p=0.1212$), however, the study mice lived significantly longer (Student's T-test, $p<0.0001$) than the documented time after IC inoculation with a 1:100 dilution of infected elk

brain (118 ± 6 DPI), suggesting a lower infectious dose present in the fecal samples.²⁷ The study mice lived an average of 214 days, with a large variability in survival days (± 87 days).

Discussion

The continued spread of CWD is of concern to the health of both wild and captive cervid populations. Indirect transmission through the environment has been demonstrated in captive animals living in paddocks where CWD-positive animals had lived³, and is a particular challenge due to the long persistence of CWD within the environment.^{7,28} Infectious material can be deposited in the environment by the decay of infected carcasses, from urine, feces, and saliva^{5,6,29}, and the spread of infected material may be aided by scavengers and predators. In this study we illustrated the ability of coyotes to pass infectivity in their feces after the ingestion of CWD-infected brain homogenate.

Coyotes have the ability to travel significant distances. This distance, however, is based upon social structure, which is generally placed in two categories; resident or transient.³⁰ Resident animals are those that utilize a specific territory and are comprised of a mated pair and sometimes pups from a previous year, while transient animals are individuals that are nomadic, more commonly male, and have no affinity for a specific territory.³⁰ In a study evaluating the range of coyotes in southern Colorado, transient animals, which represented 22% of the population, ranged over 106.5 ± 27 km², versus resident groups which ranged over 11.3 ± 5.8 km.^{2,30} Transient coyotes are therefore provided an opportunity to translocate disease to previously CWD-negative localities.

Control coyotes readily consumed the homogenized elk brain. Of the treatment coyotes, which were moved indoors two days prior to the initiation of the study, only one (#135) immediately

ate the brain homogenate. The other coyotes required supplementation with diced, raw chicken, or fish-flavored soft cat food. Although the numbers are too small to come to any definitive conclusions, it is interesting to note that the coyote that ingested the brain homogenate without chicken or cat food supplementation did not appear to transfer infectivity to any of the mice in the bioassay. Neither age nor sex appeared to have any effect on fecal shedding. However, it is possible that individual variation within the stomach environment, such as pH and flora could have influenced the passage of the infectious prions through the gastrointestinal tract.

Our experimental design was based on detection of CWD in coyote feces by PMCA prior to initiation of the bioassay. PMCA was able to repeatedly detect the presence of proteinase K-resistant prions signal in feces from DPI 1, so the bioassay was designed to evaluate feces for two days following, to account for any uncertainty in prion detection in feces. Results from the bioassay showed transmission of disease to 2/4 mouse groups in DPI 3, suggesting that infectivity may continue to be present in the feces more than three days after ingestion. We were unable to go back and increase the bioassay to include DPI 4 and 5, due to logistical reasons.

The 50 mL oral dose ingested by coyotes in this study was comprised solely of infected brain tissue and represented a high dose. In the wild, coyotes would opportunistically consume a wide variety of tissues from a kill or scavenged deer or elk carcass, likely making their actual ingested infective dose much smaller. This study was not designed to mimic a naturally consumed dose of CWD, but rather as a proof of concept to determine if infectivity could pass into coyote feces.

The passage of disease in feces is a common route of translocation for many viral, bacterial and parasitic diseases.

The results of this bioassay indicate that infectious CWD prions are able to be passed in the feces of coyotes fed infected elk brain homogenate for at least three DPI, making them a potential vector for CWD prion transport and contamination within the environment.

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Accepted Manuscript

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Table 1. Coyote number, sex, age and treatment group.

Coyote Number	Sex	Age (yrs)	Treatment Group
132	Male	6	CWD Negative Brain
134	Female	10	CWD Negative Brain
133	Male	10	CWD Positive Brain
135	Female	2	CWD Positive Brain
136	Female	6	CWD Positive Brain
137	Female	2	CWD Positive Brain

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Table 2. Transgenic mouse bioassay results. The number of mice that died from CWD/total number of mice intracerebrally inoculated per group with coyote feces. Pre-ingestion indicates fecal samples collected prior to oral ingestion of CWD-positive elk brain homogenate, and days 1, 2, and 3 after ingestion. Day 1 post-ingestion resulted in 23% of the mice becoming terminally ill, day 2, 38% and day 3, 38%.

Coyote Number	CWD Status	Pre- Ingestion	Day 1 Post Ingestion	Day 2 Post Ingestion	Day 3 Post Ingestion
132	Control	0/3	0/3	0/3	0/3
134	Control	0/3	0/3	0/3	0/3
133	CWD	0/3	1/3	0/4	0/4
135	CWD	0/3	0/4	0/4	0/4
136	CWD	0/3	0/3	3/4	3/3
137	CWD	0/3	2/2	3/4	2/2

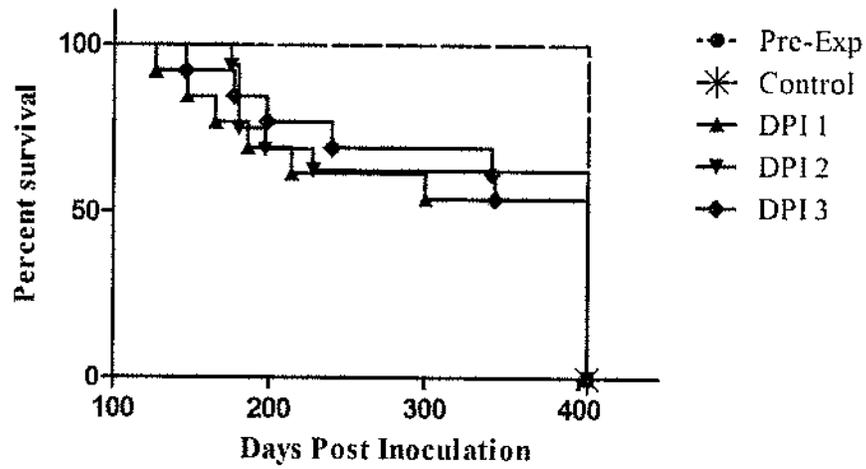


Figure 1. Coyote lymph node immunohistochemistry. Images are a representation of findings. A. CWD-positive control elk retropharyngeal lymph node. Control coyote B, and treatment coyote C, retropharyngeal lymph node. 20X magnification.

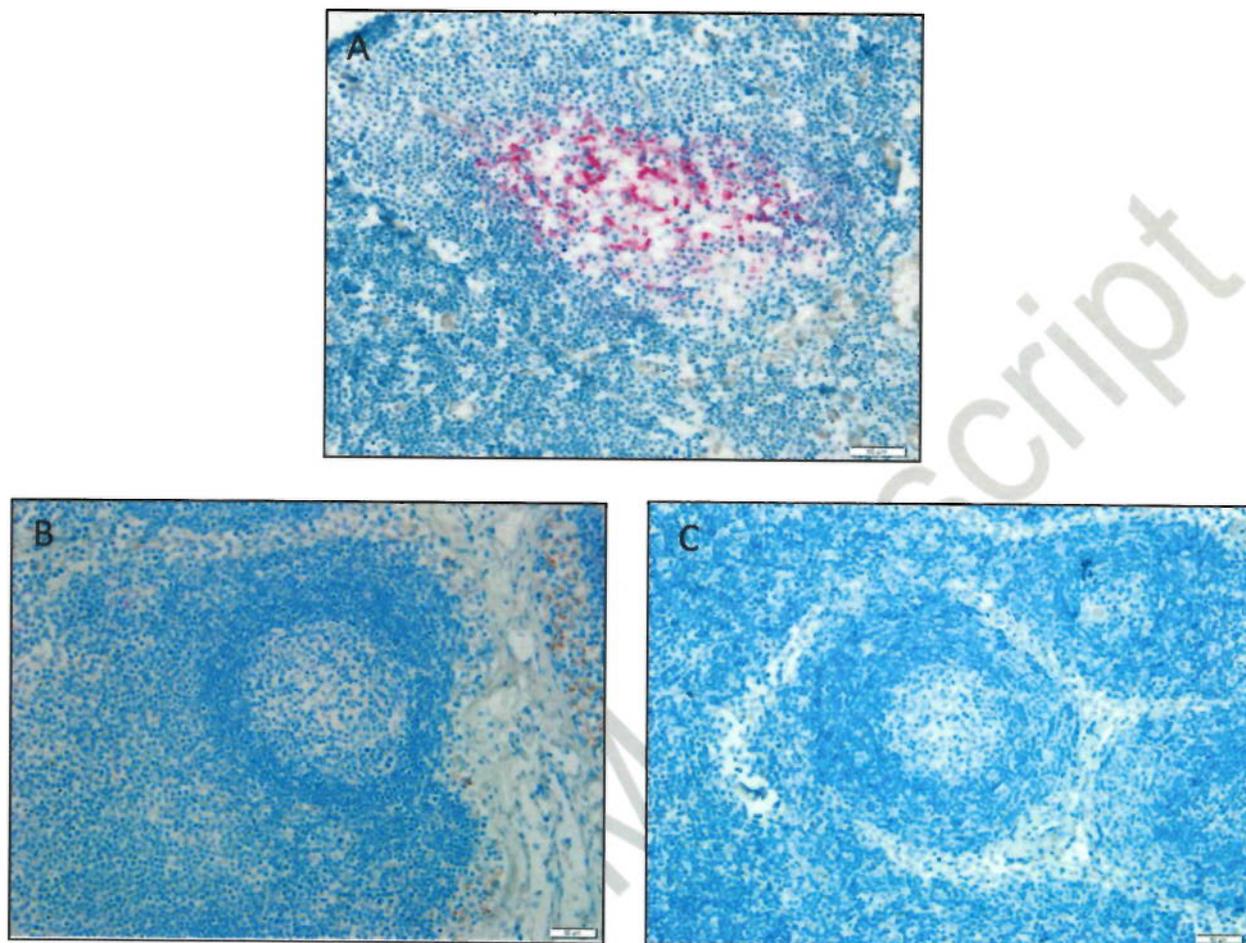


Figure 2. Transgenic mouse bioassay of coyote feces. All control mice and mice inoculated with feces collected prior to ingestion of CWD-positive elk brain remained disease-free for the duration of the study. Deaths occurred in all of the DPI tested, however, disease penetrance was incomplete. Mice inoculated with DPI 3 feces lived slightly longer than DPI 1 and 2. Each DPI group represented above combines survival times of mice from each of the study coyotes.