June 17, 2022

Lloyd Knight
Rules Review Coordinator
Idaho State Department of Agriculture
PO Box 7249
Boise, Idaho 83707

RE: Negotiated Rulemaking for Rules Governing Domestic Cervidae (IDAPA 02.04.19)

Dear Mr. Knight,

Idaho Wildlife Federation (IWF) appreciates the opportunity to provide comments on the negotiated rulemaking for the Rules Governing Domestic Cervidae (IDAPA 02.04.19).

IWF is Idaho's oldest statewide conservation organization, founded by sportsmen and women in 1936. Today, we represent a nonpartisan voice of 28 affiliate organizations with 45,000 affiliate members and individual supporters who desire to sustain and enhance Idaho's fish and wildlife, conserve their habitat, and maximize sporting opportunity for current and future generations. Our efforts advance "made in Idaho" solutions to the modern challenges of wildlife management.

We thank the Idaho State Department of Agriculture (ISDA) for reviewing IWF's petition and facilitating the two negotiated rulemaking meetings. We also appreciate the robust stakeholder participation during the two meetings. IWF supports the proposed language as submitted in our petition, which, if adopted, would increase chronic wasting disease (CWD) testing for all domestic elk and reindeer at a facility within twenty-five (25) miles from a confirmed case of CWD in wild cervids. We provide our additional comments below.

Chronic Wasting Disease Overview
CWD is an infectious disease of cervids caused by misfolded prions transmitted by ingestion of prions from contaminated environmental components or directly from contact with infected animals. The disease has a long incubation period and a long period of prion shedding. CWD is

always fatal in cervids, cannot be treated or controlled with conventional measures, and has no known cure[1].

CWD is density and frequency-of-contact dependent with both animal-to-animal transmission and environmental contamination serving as prion pathways[2]. Dispersal may enhance the spread of CWD to far greater distances than typical migration. Anthropogenic factors are the artificial translocation and the congregation of cervids, including long-distance movement and placement in high-fence operations or artificial movement of animals due to management decisions such as winter feeding, rehabilitation permits, and relocations[3].

Once CWD prions are on the landscape, it is considered improbable they will be removed. CWD prions also appear to remain infectious in carcasses for ≥ two years. Wildlife managers have concluded that CWD management actions were too little, too late, too restricted, too passive, or of insufficient duration to be successful[4]. Studies have detected prion shedding as early as 3 months after CWD exposure and sustained shedding throughout the disease course[5]. Given the average course of infection and daily production of those body fluids, an infected deer would shed thousands of prion infectious doses over the course of CWD infection[6]. Researchers concluded that "the direct and indirect environmental impacts of this magnitude of prion shedding on cervid and noncervid species are surely significant."

CWD was detected for the first time in Idaho in two hunter-harvested mule deer bucks in the Slate Creek drainage of GMU 14 in late 2021. Additional samples collected through hunter harvest detected two CWD-positive white-tailed deer, one buck and one doe, both in Unit 14. Two more suspect animals in GMU 14 tested positive for CWD, one whitetail buck and one cow elk. Since initial detection, Idaho Department of Fish & Game (IDFG) designated both GMUs 14 and 15 as a CWD Management Zone and implemented mandatory CWD testing requirements for these units. Moving forward, all harvested deer, elk, and moose in these GMUs must be tested for CWD.

Rules Governing Domestic Cervidae

IWF participated in ISDAs 2021 rulemaking meetings for Rules Governing Domestic Cervidae, and testified in support for the adoption of the rules in front of the Idaho legislature. Language from the newly adopted rules reads:

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[1] Idaho Department of Fish & Game. 2021 Strategy for Chronic Wasting Disease. p.1. https://idfg.idaho.gov/sites/default/files/cwd-strategy-2021.pdf

[2] *Ibid*. p.1

[3] *Ibid*. p.2

[4] *Ibid*. p.2

[5] Henderson, D.M., Denkers, N.D., Hoover, C.E., Garbino, N., Mathiason, C.K., and E.A. Hoover. 2015. Longitudinal Detection of Prion Shedding in Saliva and Urine by Chronis Wasting Disease- Infected Deer by Real-Time Quaking –Induced Conversion. Journal of Virology 89 (18): 9338-47.

[6] *Ibid*.

"Brain tissue from one hundred percent (100%) of all domestic elk and reindeer sixteen (16) months of age or older that die for any reason on a facility will be required to be tested for CWD for a period of sixty (60) months under the following conditions:

- A facility has imported cervids from a location within twenty-five (25) miles from a confirmed case of CWD in wild cervids
- A facility has received cervids via intrastate movement from a facility under enhanced CWD surveillance requirements at the time of transfer."

Since participating in the rulemaking process, the severity of CWD on the landscape has changed, with the first detections occurring in the Fall of 2021. The current rules only require enhanced surveillance and testing related to interstate transport and therefore CWD originating from outside of Idaho's borders. IWF believes these current rules do not consider the threat of CWD transmission from wild animals already in Idaho into domestic facilities, as well as the potential for intrastate movement of domestic cervids in areas with CWD present. Artificial congregation and movement of domestic cervids, as well as interaction between wild and domestic cervids (ingress and egress) will continue to facilitate the spread of CWD. We believe it is necessary for ISDA to increase testing requirements now that CWD is present within Idaho's borders. Therefore, IWF is overall supportive of the language submitted in the petition.

Responses to Concerns Raised by Stakeholders

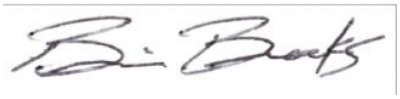
IWF appreciates the feedback we received from stakeholders in the negotiated rulemaking meetings in May and June. We remain committed to our advocacy to prevent CWD within Idaho's borders to the greatest extent possible, but understand this may come at an increased expense or burden to stakeholders. We believe our petition would align testing requirements for animals within 25-miles of a confirmed case in the wild with the 25-mile radius language taken from ISDA's language from the 2021 rulemaking process for interstate transport. However, if stakeholders have suggested changes to requirements as it relates to the distance from a confirmed case in the wild, as well as the testing percentage requirements, IWF is open to negotiation. We understand that stakeholders feel that 100% testing may not be attainable, so we are open to flexibility in requirements and the use of waivers in limited instances if managers feel it adequately addresses the current threat of CWD on the landscape. However, it should be noted that prior to 2014, ISDA required 100% testing for CWD for all animals that die, regardless of the cause.

Sportsmen and women also feel the additional burdens and financial investments has IDFG ramps up CWD testing for wild cervids. Evidence from Wisconsin shows that hunting license sales fell sharply after CWD was found in 2002 and has remained about a 5% decline. That decline in hunter participation and associated license sales decline not only impacted local communities but also the state wildlife agency. Wisconsin has now spent more than $49 million fighting CWD. Other states are grappling with similar declines in hunter participation and negative perceptions on hunting in areas with known CWD presence. Ultimately, we feel that it will take investments from both stakeholders from wild and cervid industries to tackle this threat head on. We hope to take steps with industry stakeholders today before it is too little, too late. IWF hopes that we can work together to gain support for actions such as the Chronic Wasting Disease Research and Management Act to bring critical funding for management actions to our state's wildlife and agriculture departments. IWF has also advocated for a statewide CWD

Advisory Group and hope that, if developed, industry stakeholders can work with the sporting public to find solutions for our state.

We would like to thank the Department, and especially Dr. Scott Leibsle, for meeting these increasing challenges head on with all stakeholders, and for the opportunity to participate in each of the stakeholder meetings. It has been a pleasure for our organization and we look forward to our future engagement with the Department.

Garret Visser
Conservation Program Coordinator
Idaho Wildlife Federation



Brian Brooks
Executive Director
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IDAHO CONSERVATION LEAGUE

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Lloyd Knight
Rules Review Coordinator
Idaho State Department of Agriculture
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Boise, ID 83712

June 17 , 2022

Re: Negotiated Rulemaking for the Governing Domestic Cervidae: IDAPA 02.04.19

Dear Mr.. Knight:

Thank you for considering our comments on the negotiated rulemaking for the Rules Governing Domestic Cervidae: IDAPA 02.04.19.

Since 1973, the Idaho Conservation League has had a long history of involvement with Idaho's environmental issues. As Idaho's largest state-based conservation organization we represent over 50,000 supporters who have a deep personal interest in ensuring that our natural resources are protected throughout the state. The Idaho Conservation League (ICL) seeks to minimize the risk of spread of Chronic Wasting Disease (CWD) within wild, as well as domestic cervidae, and to combat this disease as effectively as possible.

We thank the Idaho State Department of Agriculture (ISDA) for considering this rulemaking and for the opportunity to submit comments. Please feel free to contact us if you have any questions or require additional information.

Sincerely.

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General Support for the Proposed Language

ICL would like to express general support for the proposed changes brought forth by the Idaho Wildlife Federation (IWF) and their petition. Including 100% testing of domestic elk and reindeer at a facility within twenty-five (25) miles from a confirmed case of CWD in wild cervids is a prudent measure that is in alignment with CDW testing requirement for imported animals.

During the two public meetings ISDA held on this matter, some Idaho elk producers opposed the proposed testing language primarily citing concerns of economic hardship and unnecessary over-regulation. As to the issue of economic hardship, it is ICLs understanding that CWD tests cost approximately $30-35 dollars per test, while hunts for premiere bulk elk can fetch values as high as $16,000 dollars, with smaller and female elk going for ~$5-6,000. ICL understands any rulemaking that would increase economic hardship to those affected must go through an economic evaluation, and we encourage you to include this as part of the rulemaking record. While the time and effort to collect brain stem sampling for CWD (including training, record keeping, etc.) should also be factored into the costs to domestic elk producers, it would stand to reason the comprehensive cost for increased testing likely would not prove to be an overburden in comparison to its benefits. ICL anticipates any economic evaluation conducted by ISDA will likely bear this out.

As to the issue of unnecessary over-regulation, numerous studies (including those posted to the ISDA webpage for this rulemaking) highlight the importance of minimizing the spread of CWD in order to limit wild cervidae population decline. As stated within a 2017 research article, “Chronic wasting disease is difficult or impossible to eradicate with current tools, given significant environmental contamination, and at present our best recommendation for control of this disease is to minimize spread to new areas and naïve cervid populations” (DeVivo MT et. al., 2017). Implementation of the proposed testing regulations would help to detect CWD in domestic elk populations which could (and perhaps already are) serving as vectors for CWD transmission to and between wild cervidae populations due to wild population ingress and egress that is well documented by the IWF. Based on these facts, it would seem reasonable to assume that the proposed testing regulations are not an example of over-regulation but a reasonable and prudent measure to reduce the risk of CWD spread in Idaho before it gets worse.

Incubation Period of CWD and its Effects on Transmission.

ICL would like to formally submit the following study on CWD transmission; Longitudinal Detection of Prion Shedding in Saliva and Urine by Chronic Wasting Disease-Infected Deer by Real-Time QuakingInduced Conversion submitted to the Journal of Virology in 2015 authored by Henderson et. al. (see reference below). As stated in the study, “We (the researchers) detected prion shedding as early as 3 months after CWD exposure and sustained shedding throughout the disease course. Given the average course of infection and daily production of these body fluids, an infected deer would shed thousands of prion infectious doses over the course of CWD infection” (Henderson MD et. al., 2015). This study also documented and ranked the severity of

observable CWD symptoms in infected and prion shedding individuals. Their findings suggest that infectious prion shedding occurs when little to no observable symptoms are present. This obviously highlights the benefits increased CWD testing of domestic elk would likely have.

Overall Decline in Domestic Elk CWD Testing.

According to public records obtained by ICL from the ISDA, overall testing rates of domestic elk within Idaho decreased from 52% in 2017 to 46% in 2018 to 25% in 2019, and finally to 23% in 2020. In addition, public ISDA records show numerous instances of testing exemption requests being filed months after the death of individual elk, as opposed to the current 48 hour notification requirement. The decrease in overall CWD testing and improperly filed testing exemptions are concerning as they have occurred during the time leading up to when CWD was first spreading and officially reported among wild cervidae populations in Idaho in late 2021. As part of this rulemaking, we specifically request ISDA to disclose the overall rate of Elk CWD testing in 2021, and to provide information on the number of CWD Sample Submission Waiver Requests processed in 2021.

Procedures and Needed Justification to Enact a Temporary Rule.

During the June 14th negotiated rulemaking meeting, ISDA stated that at this time any inclusion of the proposed testing language, or a variation, would likely not be enacted through an immediate temporary rule and instead would not be adopted until the adjournment of the 2023 Idaho legislative session. A temporary rule is appropriate when time is of the essence and a situation calls for immediate action that cannot be delayed, and from a statutory perspective would "protect[]...public health, safety, or welfare" (Idaho Code 67-5226). As the above points and studies illustrate, CWD has already been detected in Idaho and the most effective path to combat CWD is to monitor populations and to limit its spread as best and as quickly as possible. As such, ICL requests that the ISDA reconsider their decision to forego promulgation of a temporary rule, and to justify why the ISDA is not considering the proposed testing language as a temporary rule?

Formal Support and Incorporation by Reference of the Idaho Wildlife Federation's Comment

ICL offers its formal support to and incorporation by reference of the comments submitted by the IWF on this rulemaking.

References

DeVivo MT, Edmunds DR, Kauffman MJ, Schumaker BA, Binfet J, Kreeger TJ, et al. (2017) Endemic chronic wasting disease causes mule deer population decline in Wyoming. PLoS ONE 12 (10): e0186512. https://doi.org/10.1371/journal. Pone.0186512

Henderson DM, Denkers ND, Hoover CE, Garbino N, Mathiason CK, Hoover EA. 2015. Longitudinal detection of prion shedding in saliva and urine by chronic wasting disease-infected deer by real-time quaking-induced conversion. J Virol 89:9338 –9347. doi:10.1128/JVI.01118-15.

North American Elk Breeders Association

DEVELOPING & PROMOTING THE NORTH AMERICAN ELK INDUSTRY

Date: June 16, 2022
To: Idaho State Department of Agriculture
From: Travis Lowe
Executive Director, North American Elk Breeders Association
Re: Opposition to Proposed Rules- IDAPA 02.04.19

The North American Elk Breeders Association appreciates the opportunity to submit written remarks to the Idaho State Department of Agriculture related to proposed rules governing domestic cervidae.

Since 1990, the North American Elk Breeders Association (NAEBA) serves as the trade association for elk ranches in the United States, Canada and Mexico. NAEBA is deeply involved in animal health policy and has held a seat on the United States Animal Health Association Board of Directors for over 25 years. In addition to serving as Executive Director of NAEBA, I serve as an industry representative on the Chronic Wasting Disease Working Groups for USDA APHIS.

On behalf of our members residing in Idaho, with due respect to the Department and petitioners, NAEBA stands in opposition to the proposed changes. NAEBA supports a partnership approach with agencies and stakeholders to prevent Chronic Wasting Disease but in a way that does not create precedents of new regulation, along with unfunded mandates. Our testimony illustrates our concern in greater detail.

Unprecedented Proposal. NAEBA is not aware of a state where domestic elk or other cervid producers are required to test their herd mortalities at a different rate because of Chronic Wasting Disease discovery in free-ranging deer population in proximity to their ranch. NAEBA believes deeply in having consistent rules from state to state as much as possible. This is a major reason why the industry sees value in the Federal Chronic Wasting Disease Rule located in Federal Code of Regulations. Regretfully, each time a state agency increases a threshold or creates a new requirement, it makes rules more inconsistent from state to state and harder for producers to understand how to stay in compliance.

Lack of Science- 25 Miles is Arbitrary. NAEBA is not aware of any peer-reviewed science that supports a specific 25 mile zone requirement, as proposed by the petitioners, and especially applicable to elk. NAEBA believes all rules governing Chronic Wasting Disease should be based off science and not chosen with random numbers that cannot be defended.

Low Infection Rates for Positive Farmed Elk Herds Contradicts Random Proximity Risk. In the rare circumstance a farmed elk herd becomes positive for Chronic Wasting Disease, investigations in 2020 and 2021 have shown a very low, if any at all, infection rate after depopulation. Examples seen in several states show farmed elk herds, large and small, ranging in herd size from 23 to 317 animals to have infection rates of 0%, 0.004% and 0.006%. This means animals residing within an infected herd that are literally sharing feed and water sources and in daily physical contact have very little spread. This makes it unlikely that a free-ranging discovery 24 or 25 miles has infected the farmed elk herd.

Candidly, if this proposal is adopted, it insinuates the free-ranging threat to farmed herds is very high. If this is the case, given free-ranging Chronic Wasting Disease exists in the majority of states and in ten more states than discovered in farmed herds, there should be a different conversation at play about the nature, spread and regulation of Chronic Wasting Disease.

Unfunded Mandate. The proposal requires a ranch pay for increased Chronic Wasting Disease testing out of pocket. NAEBA, in general, opposes all unfunded mandates. Today's economic climate sees skyrocketing costs to herd owners, from feed, supplies and animal health care costs. Our producers do not want to bear the cost of extra testing when they do not have a known problem in their herd. NAEBA is also concerned this proposal is being considered and may be advanced without an economic impact statement illustrating the cost to producers as implemented in present day and if other producers across the state become subject to its requirements.

Questionable Purpose. Petitioners contend this proposal aims to help monitor the Chronic Wasting Disease status of Idaho animals on both sides of the fence. However, this proposal means an Idaho ranch subject to this rule could import animals from a different state that are quickly harvested and then must be tested at the herd owner expense. What would testing out of state elk tell us about the evolving threat of free-ranging Chronic Wasting Disease in Idaho? Or even the Idaho ranch? No science exists to suggest elk imports can be infected and incubated to become positive that quickly.

Selected Science. Petitioners have submitted scientific studies for the record but these do not appear to be applicable to elk. As noted in the public meeting, it is well known in the animal health community that Chronic Wasting Disease is different in different cervid species, including susceptibility and incubation, certainly acknowledged by USDA APHIS. As an example, some cervid species raised across the country are known not to be susceptible to Chronic Wasting Disease and not regulated by USDA APHIS for that reason. NAEBA does not believe it is good policy to make broad assumptions using different species and that approach has been repeatedly rejected in reform efforts by the USDA. As previously noted, NAEBA requests rules be based off peer-reviewed science applicable to the elk industry.

The purpose of NAEBA's remarks is not to assign blame or ignore the threat of Chronic Wasting Disease. NAEBA, along with the local Idaho elk industry, desires a positive working relationship with the agency and broad stakeholders. NAEBA shares the concern of local Chronic Wasting Disease discovery. We just do not feel extra regulation, without science, is the answer, particularly with what has been learned about Chronic Wasting Disease over the last forty years. There may be a few studies based on modeling that insist Chronic Wasting Disease will destroy free-ranging herds but agencies reports in endemic states do not appear to agree. As an example, agency websites in Colorado, the first state known to have Chronic Wasting Disease, show the free-ranging elk population increased 11% from 2015 to 2021, free-ranging moose population up 28% over that same period with free-ranging whitetail holding along an average. There are many other known threats to free-ranging herds. Sadly in reality, collectively across the continent, state and federal agency rules have killed more farmed elk than Chronic Wasting Disease itself. We must recalibrate our thinking and find a better way.

We respectfully ask the Department to vote this proposal down. NAEBA is happy to participate in other discussions with stakeholders.

Respectfully submitted,

Travis Lowe
Executive Director
North American Elk Breeders Association

PLOS ONE

RESEARCH ARTICLE

Chronic Wasting Disease Drives Population Decline of White-Tailed Deer

David R. Edmunds[1¤a]*, Matthew J. Kauffman[2], Brant A. Schumaker[1], Frederick G. Lindzey[2¤b], Walter E. Cook[3¤c], Terry J. Kreeger[4¤d], Ronald G. Grogan[1¤e], Todd E. Cornish[1]

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Citation: Edmunds DR, Kauffman MJ, Schumaker BA, Lindzey FG, Cook WE, Kreeger TJ, et al. (2016) Chronic Wasting Disease Drives Population Decline of White-Tailed Deer. PLoS ONE 11(8): e0161127. doi:10.1371/journal.pone.0161127

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was primarily supported by the Morris Animal Foundation (www.morrisanimalfoundation.org) through grant numbers: Established Investigator = D07ZO-159 (TEC, DRE, MJK, FGL, WEC, and TJK), and Fellowship Training Grant = D07ZO-425 (funded DRE's graduate research stipend). The United States Geological Survey (www.usgs.gov), Wyoming Game and Fish Department (https://wgfd.wyo.gov), and International Association of Fish and Wildlife Agencies (www.fishwildlife.org) each jointly funded parts of this work

Abstract

Chronic wasting disease (CWD) is an invariably fatal transmissible spongiform encephalopathy of white-tailed deer, mule deer, elk, and moose. Despite a 100% fatality rate, areas of high prevalence, and increasingly expanding geographic endemic areas, little is known about the population-level effects of CWD in deer. To investigate these effects, we tested the null hypothesis that high prevalence CWD did not negatively impact white-tailed deer population sustainability. The specific objectives of the study were to monitor CWD-positive and CWD-negative white-tailed deer in a high-prevalence CWD area longitudinally via radio-telemetry and global positioning system (GPS) collars. For the two populations, we determined the following: a) demographic and disease indices, b) annual survival, and c) finite rate of population growth (λ). The CWD prevalence was higher in females (42%) than males (28.8%) and hunter harvest and clinical CWD were the most frequent causes of mortality, with CWD-positive deer over-represented in harvest and total mortalities. Survival was significantly lower for CWD-positive deer and separately by sex; CWD-positive deer were 4.5 times more likely to die annually than CWD-negative deer while bucks were 1.7 times more likely to die than does. Population λ was 0.896 (0.859–0.980), which indicated a 10.4% annual decline. We show that a chronic disease that becomes endemic in wildlife populations has the potential to be population-limiting and the strong population-level effects of CWD suggest affected populations are not sustainable at high disease prevalence under current harvest levels.

through grant number 000679 over multiple years (TEC, TJK, and DRE). The Wyoming Wildlife/ Livestock Disease Research Partnership (http://wyagric.state.wy.us/divisions/ts/sections-a-programs/wildlifelivestock-disease-research) partially funded this work through project number 5-35466 (TEC, FGL, DRE, and TJK). Whitetails Unlimited (www.whitetailsunlimited.com) also provided some support (no grant number; TEC, FGL, DRE, and TJK), and National Cattleman's Beef Association. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

In large mammals, chronic disease often manifests as having low detectability, moderate impacts on adult mortality and fecundity, and depressed population growth rates that are sensitive to changes in adult survival [1]. Chronic diseases are difficult to detect due to lack of mass mortalities, rapid population declines, or shifts in age structure [1]. Few studies have investigated population-level impacts of chronic diseases in wildlife populations, despite the recently increasing interest and emphasis of population-level effects of wildlife diseases [2]. The dearth of well-studied population-level effects of chronic diseases is worrisome given that research suggests diseases with preclinical stages rather than acute diseases are more likely to influence long-term population-level dynamics [2]. The widespread potential of population-level impacts warrants further research on chronic wildlife diseases [2].

Confounding the issue of investigating chronic diseases is the temptation of, and pressure on, managers to react to newly discovered diseases in ways that may not be optimal. Chronic disease may have minor effects on population vital rates early in a disease epidemic [3]. However, if the disease state shifts from epidemic to endemic, then vital rates may not be affected for years or decades and monitoring must be completed over an extended time-frame. Monitoring is crucial because a firm understanding of the effects of disease on population vital rates is necessary to accurately model disease dynamics and determine suitable management options [3]. Unfortunately, wildlife diseases are challenging to study because of their insidious nature, logistical difficulties, statistical challenges, and high costs [2, 3]. This is the case for chronic wasting disease of white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and moose (*Alces alces shirasi*) [4–8].

Chronic wasting disease (CWD) is a uniformly fatal, progressive neurodegenerative transmissible spongiform encephalopathy (TSE) that occurs in wild cervid populations in 21 U.S. states and two Canadian provinces [9]. The TSEs are caused by proteinase-resistant, abnormal isoforms (PrP^{res}) of normal host cellular proteins (PrP^{C}) known as prions. The causative agent of CWD is known as PrP^{CWD}.

There are few studies on population-level effects of CWD on cervid populations. One such study was conducted on a mule deer population near Boulder, Colorado, USA [10]. Deer abundance declined 45% during 1988–2006. It was believed CWD had been endemic since 1985 and was highly prevalent (males = 41%; females = 20%). The decline was attributed to high prevalence of CWD resulting in low overall adult survival (0.72).

Information suggests CWD has potential to cause population declines and possibly localized extinctions at high prevalence; however, this has not been definitively proven or observed. To address if and how CWD negatively impacts deer population dynamics, we intensively monitored a white-tailed deer population in southeastern Wyoming over a protracted time-period (2003–2010) to estimate population vital rates and model the influence of disease on population performance. We hypothesized that demographic rates are altered by CWD to an extent large enough to lower the population growth rate. The specific objectives were to monitor CWD-positive and CWD-negative white-tailed deer in a high-prevalence CWD endemic area throughout their lifespan via radio-telemetry and global positioning system (GPS) collars. We sought to determine the following for the two segments of the population: a) demographic and disease indices including CWD prevalence, causes of mortality, pregnancy and recruitment rates, b) annual survival, and c) finite rate of population growth (λ). These indicators allowed us to determine the magnitude of the effect of CWD on a free-ranging white-tailed deer population.

Materials and Methods

Anesthesia was used on all white-tailed deer that were captured and processed for enrollment into study. Deer were chemically immobilized with 0.03 mg/kg carfentanil and 0.7 mg/kg xylazine. All deer were injected subcutaneously with procaine/benzathine penicillin G combination (25,000 units/kg based on benzathine fraction, Bimeda, Le Sueur, Minnesota, USA) and intramuscularly with 1.5 mg/kg of Banamine (Intervet Inc., Merck Animal Health, Summit, New Jersey, USA). Immobilized deer were reversed with 100 mg naltrexone per 1 mg carfentanil and 2 mg/kg tolazoline and monitored until recovered and ambulatory. All animal procedures were approved through the University of Wyoming (Laramie, Wyoming, USA) Institutional Animal Care and Use Committee (Protocol #A-3216-01). Wyoming Game and Fish Department approved our Chapter 33 Capture Permit to capture the pre-determined number of white-tailed deer annually (Permit #531).

Study System

The study was conducted primarily on the VR ranch (True Ranches, Casper, Wyoming, USA) and surrounding areas southwest of Glenrock, Wyoming (42.861N 105.871W) in southern Converse County (S1 Fig). Elevation ranged from 1,700 m in the lower plains to 2,000 m in the rolling to steep foothills. Deer Creek and its tributaries were the main habitat for white-tailed deer [11, 12]. Riparian habitat was dominated by cottonwood (*Populus* sp.), boxelder (*Acer negundo*), willow (*Salix* sp.), Rocky Mountain maple (*Acer glabrum*), serviceberry (*Amelanchier* sp.), and choke cherry (*Prunus virginiana*). Agricultural crops were comprised of grass hay (*Bromus* sp., *Dactylis* sp., *Phleum* sp.) and alfalfa (*Medicago sativa*). Natural draws and breaks surrounding agricultural fields were dominated by sagebrush (*Artemisia* spp.) and grassland communities. Higher elevations supported mountain mahogany (*Cercocarpus montanus*), ponderosa pine (*Pinus ponderosa*), and juniper (*Juniperus* sp.). Availability of natural forage and agricultural crops was plentiful and not limiting. Primary predators in the area included cougars (*Puma concolor*), coyotes (*Canis latrans*), black bears (*Ursus americanus*), and golden eagles (*Aquila chrysaetos*). Predator-caused mortality was rare in this population (see below) despite all four predators being relatively common. Cougars appeared to prefer mule deer as their target species in this study area (Cornish and DeVivo, unpublished data). There were approximately 19.2 deer/linear kilometer of riparian habitat while the surrounding habitats were sparsely populated.

This area is endemic for CWD in white-tailed deer, mule deer, and elk. The prevalence of CWD in white-tailed deer harvested in the surrounding Wyoming Game and Fish Department (WGFD) hunt area (65) was 32% in 2003 and 43% in 2010, and 33% (n = 132) overall during the study period (2003–2010; WGFD, unpublished data). These prevalence estimates were obtained from CWD testing hunter-killed deer randomly sampled and testing by enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC) of retropharyngeal lymph nodes and/or obex region of the medulla oblongata. Importantly, the annual prevalence estimates vary quite dramatically due to small sample sizes using this method; however, the 33% prevalence based on an 8 year average likely is a good representation of the true population prevalence in adult ($\geq$1.5 years old) white-tailed deer. Hunt area 65 is part of the historic CWD core area of SE Wyoming that has been tested routinely for presence of CWD since 1998; first year white-tailed deer were sampled was 1999 and the prevalence was 28.6% (4/14). It is not known how long CWD has been endemic, but it likely has occurred since the 1970's. The WGFD did not actively manage for CWD in hunt area 65 during the study period other than annual surveillance of hunter-killed deer to track prevalence. The WGFD does not gather population data on white-tailed deer to set population objectives and they were hunted liberally

within the hunt area during this period; however, hunting was not used to actively manage for CWD. Conversely, mule deer were hunted conservatively during this period due to poor population performance, possibly linked to high CWD prevalence.

Field and Laboratory Methods

White-tailed deer were captured using Clover traps [13] and helicopter net-gunning (Leading Edge Aviation, Lewis, Idaho, USA; Quicksilver Air, Peyton, Colorado, USA) [14]. All marked deer were recaptured annually to test for CWD, replace collars or battery packs of GPS collars, and download data from GPS collars. Deer were chemically immobilized with 0.03 mg/kg carfentanil and 0.7 mg/kg xylazine based on Kreeger and Arnemo [15] and adjusted by T.C. Deer were fitted with either an ear tag (fawn—≤8 months) or collar containing a very high frequency (VHF) radio transmitter (Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA). A subset of deer were collared with store-on-board GPS receivers (Lotek Wireless, Inc., Newmarket, Ontario, Canada) equipped with VHF transmitters during 2006–2009. Body condition scores were assessed on a scale of 0–5 based on palpating abdominal and rump subcutaneous fat deposits. Blood samples were collected by jugular venipuncture for pregnancy testing females. Tonsil biopsies for CWD testing were performed as described by Wolfe et al. [16]. Immobilized deer were reversed with 100 mg naltrexone per 1 mg carfentanil and 2 mg/kg tolazoline and monitored until recovered and ambulatory [17]. All animal procedures were approved through the University of Wyoming (Laramie, Wyoming, USA) Institutional Animal Care and Use Committee (Protocol #A-3216-01).

All collared deer, including GPS-collared deer because we were not able to remotely download data due to store-on-board technology, were monitored by radio telemetry for mortality status at least twice per week. In the event of mortality, the site was investigated for evidence of cause of death and dead deer were subjected to complete necropsies to determine cause of death. All carcasses were subjected to thorough CWD examinations, which involved IHC examination of tonsil, retropharyngeal lymph node, and obex region of the medulla oblongata. Necropsies and laboratory testing of pertinent samples collected during necropsy were performed at the Wyoming State Veterinary Laboratory, Laramie, Wyoming, USA. Based on telemetry and GPS data, deer were classified as migratory if winter and summer ranges did not overlap and as a disperser if they irreversibly moved to and occupied an area geographically distinct and non-overlapping of natal range [18]. Analyses for these procedures have been described previously [12].

Number of fawns at side of collared deer was determined in late August 2008 and early September 2009. The location was determined for collared deer using radio-telemetry triangulation from roads. Deer were approached on foot, displaced from day beds, and presence or absence of fawns was determined by observing the does fleeing with fawns at side. Fawns were approximately 2-months old at time of recruitment determination and were no longer staying hidden separate from their dams.

Tissue samples available to test for CWD included annual tonsil biopsies and whole tonsil, retropharyngeal lymph nodes, and medulla oblongata sectioned at the obex from carcasses depending on post-mortem condition. Tissues were examined by IHC by staining for PrP^{CWD} using monoclonal antibody F99/97.6.1 [19] and hematoxylin for counter-staining as described previously [20]. One ml serum samples from all female deer were tested for pregnancy-specific protein B [21] by BioTracking LLC (Moscow, Idaho, USA) to establish pregnancy status.

Data Analyses

All statistical analyses and regression models were programmed using SAS (SAS Institute, Cary, North Carolina, USA) unless stated otherwise. We wished to determine the influence of

covariates, including CWD-status, on the probability of pregnancy, which is an important vital rate to understand the population dynamics as it relates to CWD. We used PROC LOGISTIC to perform a logistic regression analysis [22] on probability of pregnancy (event = 1) given CWD-status (CWD), age, body condition score (BCS) at time of capture, and year of capture (year). No fawn (≤8 months) deer were pregnant, so that age class was excluded from analysis. Single parameter models were generated to begin forward parameter selection; however, none of the parameters were significant. We generated the full model containing all four parameters:

$$LOGIT\ (Pregnancy) = \beta_0 + \beta_1 CWD + \beta_2 Age + \beta_3 BCS + \beta_4 Year \quad (1)$$

Pregnancy is an important vital rate used in the matrix population model, and thus we needed to determine if pregnancy varied by CWD-status to inform how to use the metric in the population matrix. Due to small sample sizes, we utilized PROC FREQ to perform Fisher's exact χ^2 analysis [23] comparing observed proportion of pregnant does to expected proportion of pregnant does by CWD-status for each capture year as an overall test for significance of CWD effects on pregnancy.

We needed to calculate fecundity estimates separately by CWD-status to determine if CWD impacted ability of does to raise fawns (a hypothesis of interest to population dynamics related to CWD) as well as to include as a vital rate in the population model. We performed a 2-group *t*-test [24] using PROC TTEST to compare average number of fawns per doe between CWD-positive and CWD-negative female deer. Recruitment was analyzed separately between 2008 and 2009. We used these same data with PROC GLIMMIX to perform a mixed model [25] given that some of the fawn counts were from the same does in 2008 and 2009. The model produced a log odds of fawn production given CWD-status, age, year, CWD x age, and CWD x year:

$$GLIMMIX\ (Fawn\ Count) = \beta_0 + \beta_1 CWD + \beta_2 Age + \beta_3 Year + \beta_4 CWD \times Age + \beta_5 CWD \times Year \quad (2)$$

To better understand what factors influence annual survival of white-tailed deer, we analyzed annual survival data using Cox proportional hazards model to examine survival differences given the following covariates: CWD-status, sex, age class, year, and migratory/dispersal status (binary) [26, 27]. We determined mortality dates as the first mortality event recorded by the GPS unit (4 hour delay) or estimated based on carcass condition from the first date of hearing a mortality signal during radio-telemetry for VHF-marked deer (4 or 6 hour delay depending on model). Deer were right censored at the date of the last relocation if lost to follow-up due to transmitter failure, dropped transmitter, or long-range dispersal and we failed to relocate with aerial telemetry. We also right censored deer killed during capture or by poachers, or that survived to the end of the study period. Deer killed legally by hunter harvest were not right censored as hunting was an integral part of the study system. Deer that initially were CWD-negative then tested CWD-positive during subsequent captures were right censored as CWD-negative at the capture date of first CWD-positive test. We tested proportionality of hazards ratios using the TEST option in PROC PHREG [28]. We utilized an Extended Cox model after we determined that proportionality of hazards ratios was not met ($Wald\ \chi^2_4 = 9.0252$, $P = 0.0605$). We used PROC PHREG in SAS to evaluate the effects of the above covariates plus age x duration interaction term (to account for lack of proportionality) on annual survival of deer, modeled as duration known alive (duration) x living status (e.g., alive (0) or dead (1);

status) [29]. The following was the full model:

$$PHREG\,(Duration \times Status) = \beta_0 + \beta_1 CWD + \beta_2 Sex + \beta_3 CWD \times Sex + \beta_4 Age + \beta_5 Age \times Log(Duration) + \beta_6 Migration + \beta_7 Dispersal + \beta_8 Year \quad (3)$$

We implemented backwards elimination for parameter selection and Akaike's Information Criteria (AIC) [30] for model selection (supported models were within 2 AIC values of the model with the lowest AIC value; ΔAIC) along with consideration of significant parameters (based on Wald χ^2 statistic) and our biological knowledge of the system.

We wished to compare subcategory-specific annual survival rates based on capture year$_t$ to capture year$_{t+1}$ to better understand the pairwise comparisons of significant factors from the Cox proportional hazards modeling. While the Cox proportional hazards modeling indicates which factors are important and the risk associated with each factor, it does not provide an actual survival estimate to be used for intra- and inter-population comparisons. Therefore, we used Kaplan-Meier survival estimation [31] to generate these metrics with PROC LIFETEST in SAS. We generated survival estimates separately by age and CWD-status for males and females. The χ^2 Log-Rank test [32] determined differences in annual survival rates by sub-categories using PROC LIFETEST by strata.

We also needed to generate age and CWD-status specific survival estimates by biological year (June 1 –May 31) for does to be used as a vital rate in the matrix population model. For these analyses, we once again used the Kaplan-Meier survival estimator. Biological year of fawns was defined as September 1 –May 31 because fawn recruitment of marked does was determined during the first week in September. However, these fawns were not marked with radio transmitters; we captured fawns when they were much older in February; therefore, we had to estimate fawns survival from September to February. We combined published estimates of fawn survival during this missing time period from Dusek et al. [33] and survival data of fawns tracked on this study from February1 through May 31 with a weighted average to account for differing lengths of time between the two sources of survival estimates to produce one fawn survival estimate.

We needed to calculate annual CWD incidence as a vital rate to be used within the matrix population model, but we also needed to perform comparisons on these estimates by sex and age class to inform how to populate the matrix with this metric. We calculated annual CWD incidence using the time-to-event (CWD conversion) Kaplan-Meier estimator [31] with PROC LIFETEST. Incidence was calculated separately by sex and for each age-class. We performed a 2-group *t*-test [24] to determine if a difference in incidence existed between bucks and does using PROC TTEST (Cochran option).

Our ultimate question of interest was to determine the impact of CWD on the growth rate of this population. We calculated the finite rate of population growth (λ) using a post-breeding, age-structured, female-only 18 x 18 dimension Leslie matrix [34, 35] in MATLAB® (The MathWorks, Inc., Natick, Massachusetts, USA). Vital rates incorporated were fecundity (average number of fawns per doe in the first week of September) and age-specific pregnancy rates, survival rates, and CWD incidence. All vital rates were estimated separately for CWD-negative and CWD-positive deer except fecundity, which did not differ by CWD-status. The 18 x 18 transition matrix, **A**, represented the estimated demographic rates of the study population with both CWD-negative and CWD-positive females, and the transition between them due to infection, represented (Fig 1). We calculated the population growth rate as the dominant eigenvalue, λ_1 [35]. To determine the population vital rates that most influenced lambda (i.e., the vital rate that lambda was most sensitive to and would change the most if those vital rates changed), we

$$\begin{pmatrix} Fawn(-) \\ Fawn(+) \\ 1(-) \\ 1(+) \\ 2(-) \\ 2(+) \\ 3(-) \\ 3(+) \\ 4(-) \\ 4(+) \\ 5(-) \\ 5(+) \\ 6(-) \\ 6(+) \\ 7(-) \\ 7(+) \\ 8(-) \\ 8(+) \end{pmatrix} \times \begin{pmatrix} 0 & 0 & \hat{\phi}_{1-}\hat{b}\left(1-\hat{P}_f\right) & \hat{\phi}_{1+}\hat{b}\left(1-\hat{P}_f\right) & \dots & \hat{\phi}_{7-}\hat{b}\left(1-\hat{P}_f\right) & \hat{\phi}_{7+}\hat{b}\left(1-\hat{P}_f\right) & \hat{\phi}_{8-}\hat{b}\left(1-\hat{P}_f\right) & \hat{\phi}_{8+}\hat{b}\left(1-\hat{P}_f\right) \\ 0 & 0 & \hat{\phi}_{1-}\hat{b}\left(\hat{P}_f\right) & \hat{\phi}_{1+}\hat{b}\left(\hat{P}_f\right) & \dots & \hat{\phi}_{7-}\hat{b}\left(\hat{P}_f\right) & \hat{\phi}_{7+}\hat{b}\left(\hat{P}_f\right) & \hat{\phi}_{8-}\hat{b}\left(\hat{P}_f\right) & \hat{\phi}_{8+}\hat{b}\left(\hat{P}_f\right) \\ \hat{\phi}_{f-}\left(1-\hat{P}_f\right) & 0 & 0 & 0 & 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ \hat{\phi}_{f-}\left(\hat{P}_f\right) & \hat{\phi}_{f+} & 0 & 0 & 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \hat{\phi}_{1-}\left(1-\hat{P}_1\right) & 0 & 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \hat{\phi}_{1-}\left(\hat{P}_1\right) & \hat{\phi}_{1+} & 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \dots\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \dots\ \dots\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ \dots\ 0\ 0\ 0\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ \dots\ \dots\ 0\ 0\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ 0\ 0\ \dots\ 0\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ 0\ 0\ \dots\ \dots\ 0\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ 0\ 0\ 0\ 0\ \dots\ 0\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ 0\ 0\ 0\ 0\ \dots\ \dots\ 0\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ \dots\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ \dots\ \dots & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0 & \hat{\phi}_{7-}\left(1-\hat{P}_7\right) & 0 & \hat{\phi}_{8-}\left(1-\hat{P}_8\right) & 0 \\ 0 & 0 & 0 & 0 & 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0 & \hat{\phi}_{7-}\left(\hat{P}_7\right) & \hat{\phi}_{7+} & \hat{\phi}_{8-}\left(\hat{P}_8\right) & \hat{\phi}_{8+} \end{pmatrix}$$

$\mathbf{n_t}$ $\mathbf{A}$

Fig 1. Leslie Matrix Population Model. Post-breeding, age-structured, female-dominated 18x18 Leslie Matrix model of white-tailed deer population on Deer Creek drainage SW of Glenrock, WY (2003–2010) that was located within the chronic wasting disease (CWD) endemic area. n_t represents the number of deer in each age class by CWD-status ((-) = PrP^{CWD} not detected, (+) = PrP^{CWD} detected). $\hat{\phi}_{i(-/+)}$ represents estimated survival by age class, *i*, and CWD-status (- or +), $\hat{b}$ is the estimated fecundity rate and $\hat{P}_i$ is the age-specific CWD incidence rate.

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performed sensitivity analysis of **A** in MATLAB using the *vitalsens.m* function developed by Morris and Doak [36] to quantify how sensitive λ was to a change in value of each vital rate. In addition, we determined the elasticity of each vital rate [35].

We calculated the 95% confidence interval for λ_1 using parametric bootstrapping [36]. Specifically, 2000 each of age and CWD-status specific survival and CWD incidence rates were randomly estimated from the β-distribution using the *betaval* function and 2000 estimates of the fecundity rate were generated using the *stretchbetaval* function; both functions were from the *popbio* package [37] in program R v.3.2.5 [38]. The estimates were based on the mean and bias-corrected variance estimate generated using the *Kendall* function in the *popbio* package. We then used the 2000 vital rate estimates to generate 2000 λ_1 estimates with the *eigen.analysis* function in the *popbio* package based on the **A** matrix and we determined the 95% confidence interval from the 0.025 and 0.975 quantiles of the λ_1 distribution [36]. The *popbio* package reproduces the same results in program R as the MATLAB-based analysis.

To understand the effect of CWD on population vital rates, we created two matrices, **Aneg** and **Apos,** taking the form of 9 x 9 transition matrices for CWD-negative and CWD-positive populations respectively. The two matrices differed in that one model assumed 0% CWD-prevalence and one model assumed 100% CWD-prevalence, allowing determination of the magnitude in change of λ ($\Delta\lambda$) due to CWD. We performed a life table response experiment (LTRE) on the transition matrices, **Aneg** and **Apos,** using the *vitalsens.m* function in MATLAB [35] to better understand the influence of CWD on λ_1.

To evaluate the influence of CWD incidence on population growth rate, we varied incidence from 0 to 1.0 by 0.05. This range of incidence rates was inserted in the full 18 x 18 matrix model, keeping all other parameters equal, and calculating λ across the range. State wildlife agencies routinely track CWD by annual prevalence from hunter-harvested or targeted deer and elk. We converted each incidence rate into annual prevalence based on the following equation:

$$\boldsymbol{P} = \frac{\boldsymbol{I} \times \overline{\boldsymbol{D}}}{\mathbf{1} + \boldsymbol{I} \times \overline{\boldsymbol{D}}} \tag{4}$$

where P was prevalence, I was incidence, and $\overline{D}$ was the estimated duration of illness estimated by Kaplan-Meier [31] using PROC LIFETEST. All CWD-positive deer were included in the analysis with the enrollment date set as the date of first positive CWD test and mean time known alive (i.e., $\overline{D}$) calculated by Kaplan-Meier analysis [31].

We were interested in the population age structure to determine if the population was shifted to a young age structure. We calculated the dominant right eigenvector ($\mathbf{w_1}$), which gives the stable population structure, using function *eigenall.m* in MATLAB [35] to determine the stable population age structure. Given the following equation:

$$\mathbf{A}\mathbf{w}_i = \lambda_i \mathbf{w}_i \tag{5}$$

where $\mathbf{w}_i$'s are age-specific contributions to population growth. When one sums the age specific $\mathbf{w}_i$'s and then divides each $\mathbf{w}_i$ by the total, the proportion of the population in each age class is determined [35]. The age structure of both male and female deer by CWD-status was determined using this method.

Results

During the study period (January 2003–February 2010), 112 deer were captured as fawns ($\leq$8 months-old; female: male = 57: 55) and 63 deer captured originally as adults ($\geq$1.5 years-old; female: male = 27: 36). All deer were recaptured annually. Overall CWD prevalence during the study period (last known CWD-status of each individual deer) was 35.4% (n = 161). Prevalence was higher in does (42%, n = 81) than bucks (28.8%, n = 80, χ^2_1 = 6.608, P = 0.01). Average annual CWD prevalence (based on annual tonsil biopsies) was 23.8% (n = 345) overall, 24.3% (n = 202) for does, and 23.1% (n = 143) for bucks.

There were 118 mortalities (CWD-negative = 64, CWD-positive = 50, CWD-unknown = 4) during the study period (S1 Table). Hunter harvest was the most common cause of mortality (n = 46) and more CWD-positive deer (n = 19) were harvested than expected based on average annual CWD prevalence (41.3% vs. 23.8%, χ^2_1 = 8.876, P = 0.029). Bucks were more common (76%) than does in the harvest. There were 20 capture-related mortalities, representing 4.2% of all captures (n = 476). This is an overestimate of capture-related mortality because many non-target deer were captured and released without injury during Clover trapping. Seventeen deer (female: male = 12: 5) died of clinical CWD; does comprised 71% of clinical cases, but made up only 48% of the study population.

Age, CWD-status, year, and body condition score did not influence pregnancy. Average proportion pregnant for CWD-negative deer was 0.95 (n = 109, 95% C.I. = 0.92–0.99) and for CWD-positive deer was 0.92 (n = 38, 95% C.I. = 0.84–1.0). There was not a statistically significant difference detected in proportion pregnant by CWD-status annually or across all years combined (χ^2_1 = 0.601, P = 0.438).

Average number of fawns per doe was 0.74 (95% C.I. = 0.47–1.00). There was no statistically significant difference detected in the average number of fawns per doe by CWD-status in 2008

(CWD-negative = 0.56, CWD-positive = 0.67, t_{17} = -0.23, P = 0.819) or 2009 (CWD-negative = 0.90, CWD-positive = 1.00, t_{13} = -0.22, P = 0.829).

Six Cox proportional hazards models were evaluated using AIC, statistical significance of parameter estimates, and biological knowledge of the system. The full model included the following parameters: CWD, Sex, CWD x Sex, Age, Age x Time, Migration, Dispersal, and Year; the top model included CWD, Sex, Age, Age x Time, and Dispersal. The most significant parameter was CWD, which had the highest hazard ratio. The CWD-positive deer were 4.51 times more likely to die annually than CWD-negative deer (β_1 = 1.51, χ^2_1 = 44.62, P <0.001, 95%, C.I. = 2.9–7.0). Bucks were 1.70 times more likely to die than does (β_2 = 0.532, χ^2_1 = 5.17, P = 0.023, 95% C.I. = 1.08–2.69). Deer that did not disperse were 1.61 times more likely to die than deer that did disperse; however, the result was not statistically significant (β_5 = -0.493, χ^2_1 = 1.118, P = 0.290, 95% C.I. = 0.656–4.08). Age and age over time did not affect survival probability.

Kaplan-Meier survival log rank tests were performed on all ages combined (overall) and by each age class (Table 1). Survival comparisons were all statistically different except CWD-positive females vs. males (χ^2_1 = 2.73, P = 0.098), indicating sex did not significantly affect survival of CWD-positive deer. Of the five significant comparisons, CWD-positive deer had lower survival than CWD-negative deer and males had lower survival than females. Log rank tests were

Table 1. Annual Survival Rate Comparisons.

Category	Results	Overall	Fawn	1.5	2.5	3.5	4.5	5.5+
Female:	Survival: CWD (-)	0.853	0.552	0.889	0.875	0.741	1.00	1.00
CWD (-) vs. (+)	Survival: CWD (+)	0.481	*n* = 1	0.400	0.500	0.500	0.500	0.500
	χ^2_1	23.49	---	8.99	0.875	4.12	5.33	1.69
	P-value	**<0.001**	---	**0.003**	0.351	**0.042**	**0.021**	0.194
Male:	Survival: CWD (-)	0.729	0.332	0.791	0.667	0.525	1.00	---
CWD (-) vs. (+)	Survival: CWD (+)	0.304	*n* = 1	0.200	0.200	0.500	0.333	---
	χ^2_1	13.53	---	9.11	6.05	0.000	4.09	---
	P-value	**0.000**	---	**0.003**	**0.014**	1.00	**0.043**	---
All deer:	Survival: CWD (-)	0.801	0.737		0.750	0.780	1.00	1.00
CWD (-) vs. (+)	Survival: CWD (+)	0.396	0.250		0.333	0.500	0.429	0.444
	χ^2_1	39.70	17.33		9.52	3.73	8.90	10.21
	P-value	**<0.001**	**<0.001**		**0.002**	0.054	**0.003**	**0.001**
All deer:	Survival: Females	0.758	0.524	0.745	0.788	0.668	0.786	---
Female vs. male	Survival: Males	0.612	0.543	0.645	0.455	0.643	0.750	---
	χ^2_1	11.96	1.14	0.514	6.03	2.03	0.520	---
	P-value	**0.001**	0.286	0.473	**0.014**	0.155	0.471	---
CWD (-):	Survival: Females	0.853	0.552	0.889	0.875	0.741	1.00	---
Female vs. male	Survival: Males	0.729	0.332	0.791	0.667	0.700	*n* = 1	---
	χ^2_1	11.03	0.671	1.39	2.99	1.64	---	---
	P-value	**0.001**	0.413	0.239	0.084	0.200	---	---
CWD (+):	Survival: Females	0.433	0.333		0.500	0.500	0.500	---
Female vs. male	Survival: Males	0.304	0.167		0.200	0.500	0.333	---
	χ^2_1	2.73	0.028		1.72	0.064	0.270	---
	P-value	0.098	0.868		0.190	0.800	0.604	---

Kaplan-Meier survival rates and log rank χ^2 test results by sex and chronic wasting disease (CWD)-status (CWD-negative = (-), CWD-positive = (+)) of white-tailed deer captured, CWD-tested annually, radio-collared, and monitored by radio-telemetry SW of Glenrock, WY (2003–2010). Results presented overall and by age-classes.

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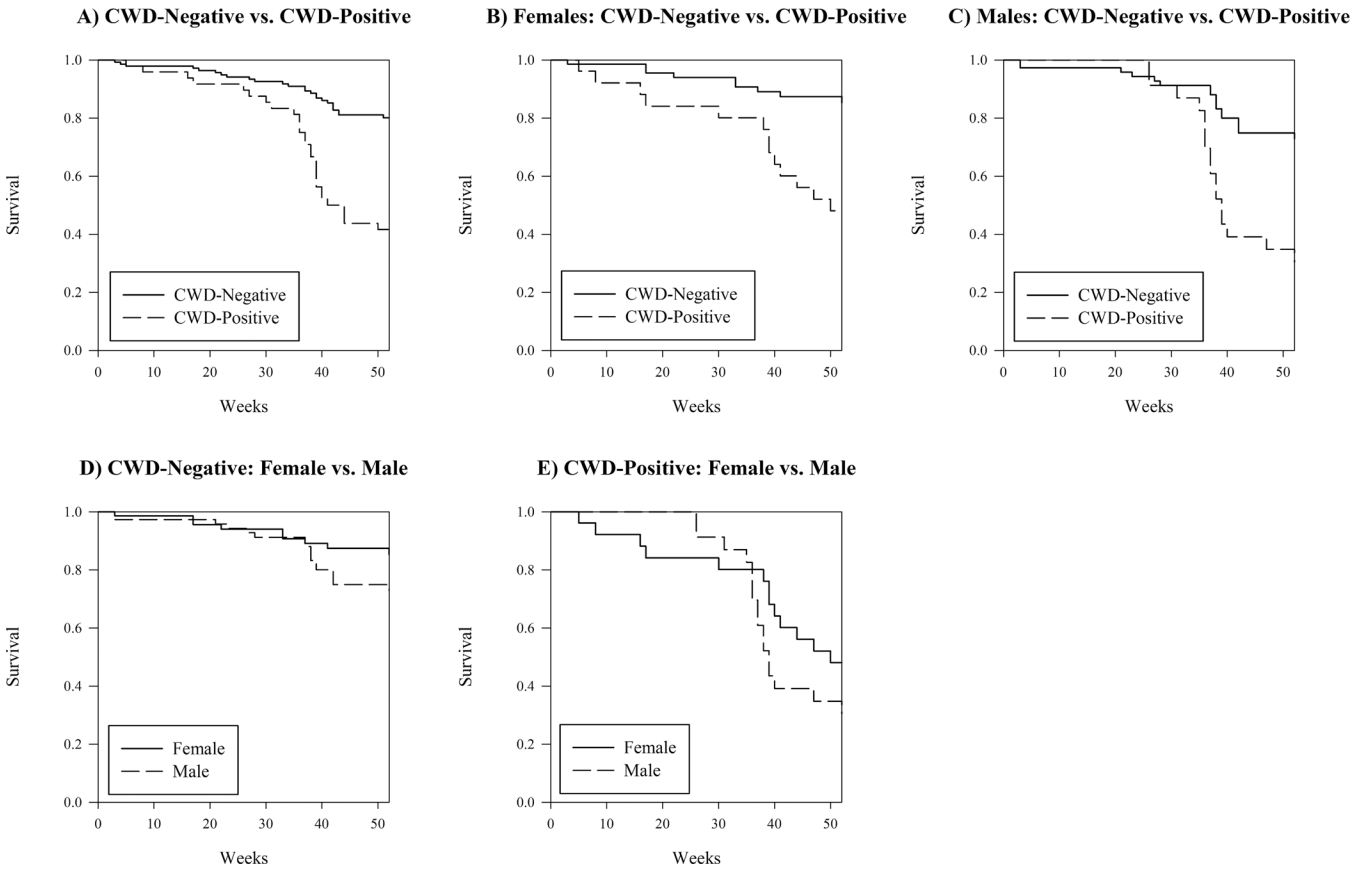

Fig 2. Kaplan-Meier Annual Survival Rate Curves. Survival rate curves of segments of the white-tailed deer study population that was captured, tested for CWD by tonsil biopsy, marked with radio transmitters, and followed by radio telemetry SW of Glenrock, WY (2003–2010).

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highly significant by CWD-status and less-so by sex, which indicated CWD was a greater indicator of annual survival. There were no clear trends by age class.

There was a large difference in annual survival of CWD-positive deer (0.396) compared to CWD-negative deer (0.801, $\chi^2_1 = 39.70$, $P = <0.001$, Fig 2A). Kaplan-Meier survival curves indicated a slow, steady drop in survival of CWD-positive does (Fig 2B). Survival estimates were similar during most of the year for CWD-positive and CWD-negative bucks and then dropped slowly for CWD-negative bucks but precipitously for CWD-positive bucks between weeks 35–40, which coincided with the 6-week hunting season. Fewer CWD-negative bucks survived annually (73%) than CWD-negative does (85%; Fig 2D). The CWD-positive deer were the only group that did not differ significantly by sex, but both survival rates were extremely low (Fig 2E, Table 1).

Annual CWD incidence increased more rapidly in bucks, reaching peak in the first year of life (51%—indicated by CWD-positive test as a 1.5 years-old (yearling)), declining slightly in the second year, returning to near peak incidence during the third year, and then declining steadily to 0 by the 6th year (Fig 3). Incidence increased slower in females, but reached a higher peak than males (65%) during the 5th year, then also dropping to 0 during the 6th year. Incidence was not significantly different between sexes ($t_4 = -1.26$, $P = 0.277$).

The dominant eigenvalue, λ_1, of our 18 x 18 matrix model was 0.896 (0.859–0.980), which indicates 10.4% annual decline from 2003–2010, assuming a stable age distribution. A λ_1 of 0.896 is not sustainable ($t_{0.5} = 5$ years, $t_{extinction} = 48$ years). To determine magnitude of $\Delta\lambda_1$

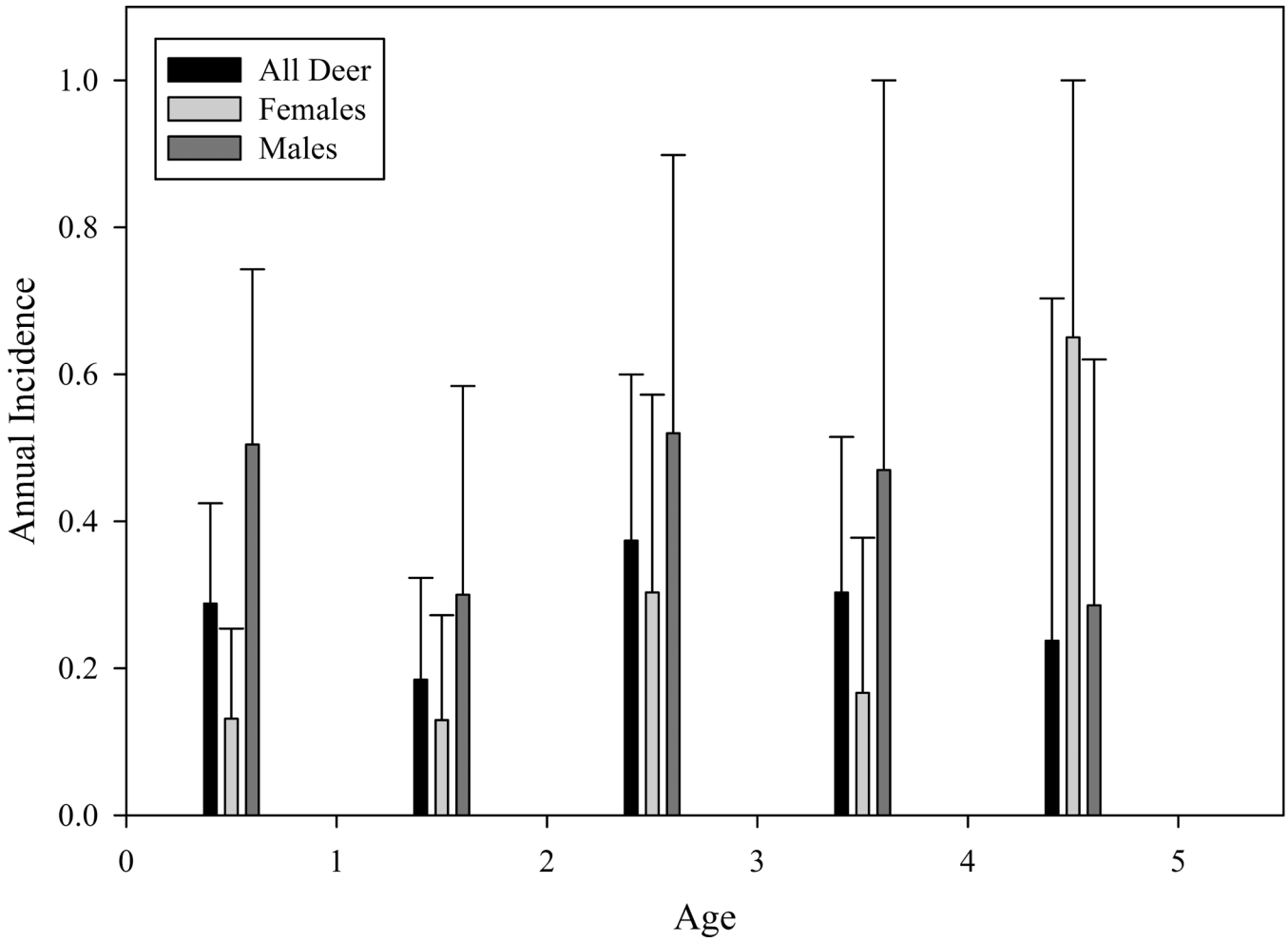

Fig 3. Annual Incidence Rates. Chronic wasting disease (CWD) annual incidence rate by sex and age class of white-tailed deer captured, CWD-tested annually, radio-collared, and monitored by radio-telemetry SW of Glenrock, WY (2003–2010). The CWD incidence was calculated by Kaplan-Meier time to event analysis.

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due to CWD, λ_1 was determined for a subpopulation of CWD-negative (**Aneg**) and CWD-positive (**Apos**) deer, which were 1.07 and 0.681 respectively. The results suggest CWD significantly depressed λ_1 in the study population.

Sensitivity analysis indicated λ_1 was most sensitive to changes in survival of CWD-negative fawns (0.280) and yearlings (0.269) and, to a lesser extent, 2.5 years-olds (0.154). Fecundity was not important; however, λ_1 was slightly sensitive to changes in fecundity of yearlings (0.156). Changes to vital rates in older age classes did not significantly affect λ_1. Elasticity results were similar to sensitivity analysis, except in the case of fecundity, which had small values ($\leq$0.06) for every age class (S2 Table).

Survival of yearlings and 2.5 years-olds was most severely reduced by CWD and had the greatest impact on lowering λ_1. These results, combined with sensitivity analysis, suggest that survival overall across younger age cohorts is influencing λ_1. The LTRE indicated that survival of yearlings and 2.5 years-olds contributed most to the change in λ_1 ($\Delta\lambda_1$) cause by CWD, with $\Delta\lambda_1$ of 0.144 and 0.173 respectively. No other vital rate caused a $\Delta\lambda_1 \geq$0.025. The LTRE was similar to sensitivity analysis except in the case of fawn survival, which contributed 0 to the

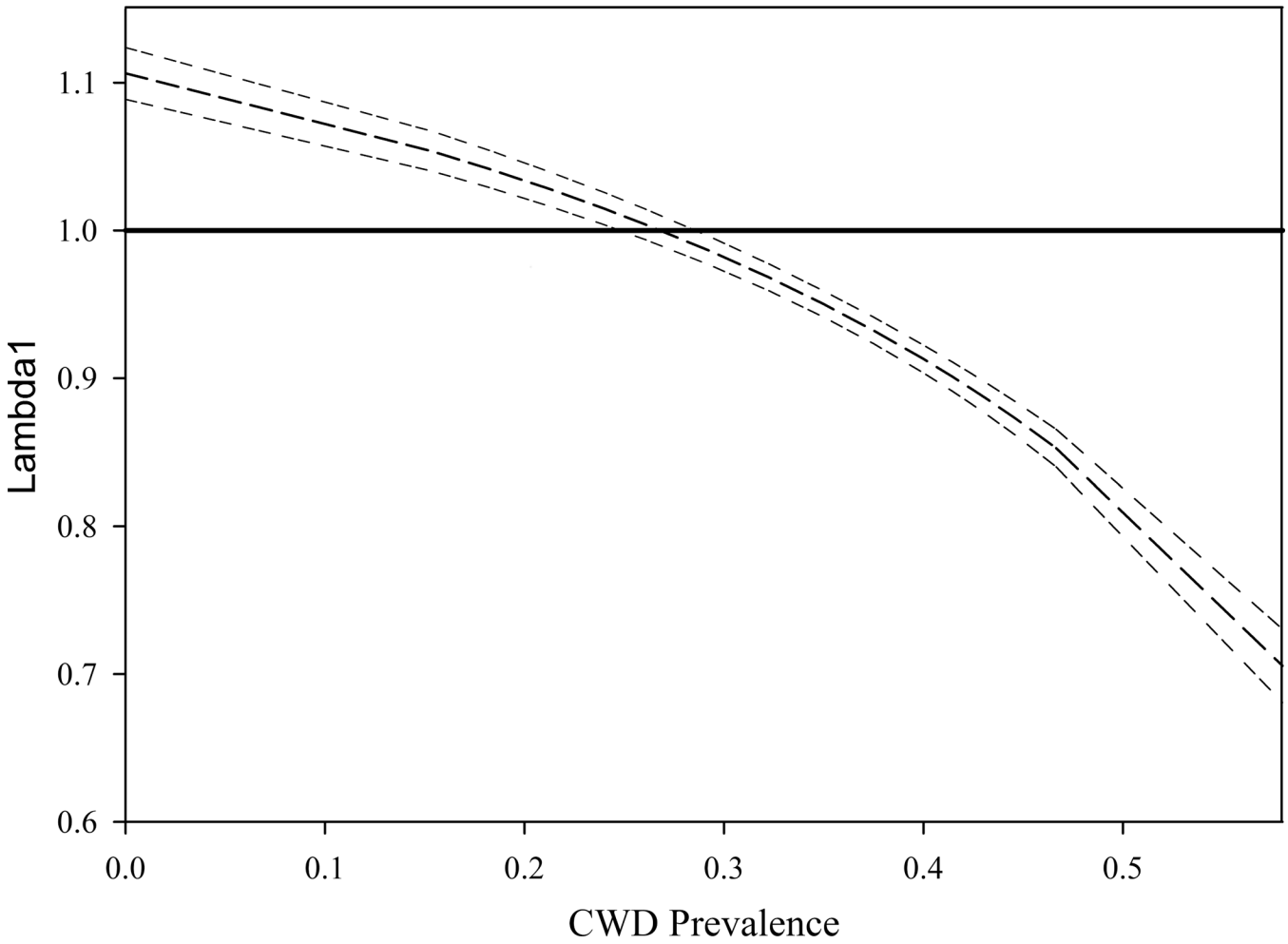

Fig 4. Lambda by Prevalence. Curvilinear relationship between increasing chronic wasting disease (CWD) prevalence and decreasing lambda (λ_1) simulated from vital rates of a white-tailed deer population captured, CWD-tested annually, marked with radio transmitters, and monitored by radio-telemetry SW of Glenrock, WY (2003–2010). The curve was generated by holding all population vital rates constant, but varying incidence up and down from the population incidence by intervals of 0.05, re-running the Leslie matrix population model with the constant vital rates and altered incidence value populating the transition matrix, **A**, and calculating lambda. The incidence rates were then converted into prevalence estimates to be more useful to wildlife managers because state wildlife agencies collect surveillance data in prevalence proportions, not incidence rates. The solid horizontal line at $\lambda_1 = 1.0$ represents the threshold at which population growth begins to decline ($\lambda_1 < 1.0$) and the dark dashed line is the simulated population growth rate with accompanying 95% confidence intervals (lighter double dashed lines).

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treatment effect because survival of CWD-negative and CWD-positive fawns was equal. Overall treatment effect of CWD was 0.348. The magnitude of the negative effect on λ_1 by CWD infection was 0.382. Survival was recalculated for each age class with all hunter-related mortalities right censored from Kaplan-Meier analysis. With hunting-related mortality censored, the resulting vital rates estimated a λ_1 of 1.00.

By varying incidence rates, recalculating λ_1 for each incidence rate and plotting λ_1 by incidence, it was determined λ_1 dropped below 1.0 at an annual incidence rate of 0.26. Transforming incidence into prevalence and then plotting the λ_1 values with the new prevalence values estimated λ_1 was <1.0 at 0.27 (27%, Fig 4).

The dominant right eigenvector, w_1, determined the proportion of CWD-negative deer was highest in the fawn and yearling age classes and continued a constant, steep downward slope until the proportion was 0.005 in 6.5 years-old deer (Fig 5). Conversely, proportion of CWD-

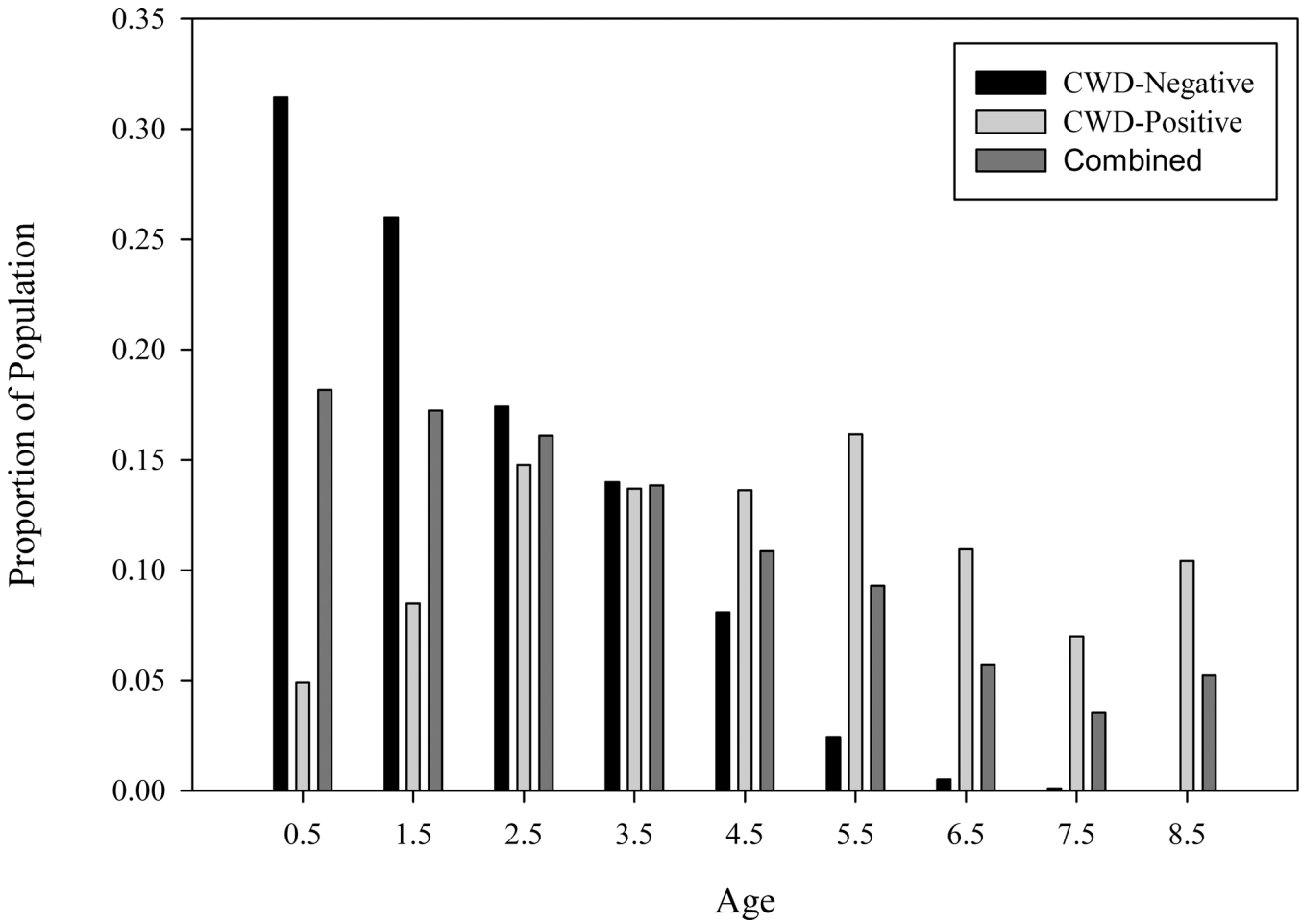

Fig 5. Population Age Structure. Proportional age structure of females separate by chronic wasting disease (CWD)-status and combined CWD-negative and CWD-positive deer of white-tailed deer captured, CWD-tested annually, marked with radio transmitter, and monitored by radio-telemetry SW of Glenrock, WY (2003–2010). Age structure was calculated from the dominant right eigenvector, w_1, of the Leslie matrix population model of the transition matrix, **A**. The CWD-negative age structure was the result of assuming 0% CWD incidence modeled with a 9x9 **ANeg** transition matrix, the CWD-positive age structure was the result of assuming 100% CWD incidence with a 9x9 **APos** transition matrix, and the combined age structure was the result of modeling what is occurring in this population currently based on the 18x18 combined transition matrix, **A**; this result represents the actual population age structure.

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positive deer was lowest in fawns and yearlings, climbed to approximately 0.15 in 2.5 years-old deer and then plateaued (Fig 5). The age structure of all deer combined showed the majority of the population was found in the first three age classes followed by a rapid decline in older age classes (Fig 5), indicating the age structure was shifted to the left and dominated by young, immature, and sub-prime-aged deer.

Discussion

The difference in survival by CWD-status and the high proportion of CWD-positive deer in this population help explain the declining population trend ($\lambda_1 = 0.896$). The CWD-positive deer were 4.5 times more likely to die annually than CWD-negative deer. These results support concerns of wildlife managers, wildlife disease experts, and conservationists that this endemic (chronic) disease can negatively impact deer population sustainability at high disease prevalence. The sensitivity analysis and LTRE indicated survival of fawns, yearlings, and 2.5 year-old

CWD-negative deer were primarily responsible for the reduction in λ_1 caused by CWD. It is likely that CWD and hunter harvest, the main causes of mortality, have produced the young age structure observed in this population. At the current λ_1, this population is not sustainable with possible extinction in 48 years at current levels of mortality and fecundity given the worst-case scenario of frequency dependent transmission [39] and no immigration or genetic selection for less susceptible genotypes for CWD [40].

Our estimate of λ is the lowest reported for a free-ranging cervid population with endemic CWD. Dulberger et al. [41] reported a λ of 0.97 (95% credible interval = 0.82–1.09) in a CWD-endemic mule deer population in Colorado, and $\lambda = 1.0$ has been reported for CWD-endemic elk populations in South Dakota and Colorado [42, 43]. These values were not particularly worrisome as λ either overlapped 1.0 given the credible interval or was equal to 1.0, indicating stable populations. It is particularly concerning how low our λ_1 value was given that the study species was white-tailed deer, which have a higher lifetime reproductive potential than the other three CWD susceptible species.

Hunter harvest often is a major cause of mortality in white-tailed deer, which are the most common and wide-spread big game species in North America. We demonstrated that CWD-positive adults were over-represented in hunter harvest, and others [44] have suggested CWD-positive mule deer also are more vulnerable to hunter harvest. The behavioral shifts, including movement patterns, changes in breeding behavior during harvest, decreased reaction time to stimuli, and changes in habitat type used by CWD-positive mule deer may have caused biased harvest proportions. Conversely, Grear et al. [45] found no difference in harvest susceptibility between CWD-negative and CWD-positive white-tailed deer in Wisconsin, perhaps due to relatively low CWD prevalence (6.3% in adults). It is probable that the behavioral changes suggested by Conner et al. [44] affect CWD-positive deer susceptibility to harvest. Captive CWD-positive deer often show altered response to human activity [4], including an apparent lack of recognition of human presence. Activity analysis suggested CWD-positive bucks did not participate in the rut at the same level as CWD-negative bucks; the rut coincided with the hunting season [11]. Our data support the notion that CWD-positive bucks were less aware of the rut and the hunting season and were more susceptible to being shot by a hunter.

Over-representation of CWD-positive deer in the hunter harvest suggests behavior is altered by CWD prior to clinically recognizable CWD infection. Rather than thinking of CWD as a strictly pre-clinical disease followed by a short, obvious clinical stage of disease, we believe CWD infection should be envisioned as a slow, progressive decline in health and alteration of normal behavior, which ends with clinically recognizable disease. Given the relatively short clinical stage of CWD and the limited hunting season, it is hard to believe CWD-positive deer would be more susceptible to harvest if this slow alteration in health and behavior does not occur. Further, the majority of hunters do not intentionally harvest emaciated or sick animals.

There was a discrepancy in sex ratio of deer that died of clinical CWD (female: male = 12: 5). The high proportion of bucks in the harvest (76%) and over-representation of CWD-positive deer compared to CWD-negative deer may explain why females comprised 71% of clinical CWD cases. Data suggest CWD-positive bucks were harvested at a higher rate than expected and prior to reaching terminal stages of disease while the low harvest rate of does facilitated disease progression to clinical CWD. Females lived longer (137.2 weeks) after testing positive for CWD than bucks (107.4 weeks), which supports this argument. Also, the matriarchal social structure of females may explain why CWD incidence was higher in females and a more steady progression than males. Males were removed earlier in disease progression and had less time to spread disease directly to susceptible bucks in their bachelor herds throughout most of the year. Meanwhile, females progressed to clinical CWD, presumably shedding infectious prions into the environment and transmitting prions directly to susceptible females in their familial

groups early in infection [46] and throughout most of the year. It is known that CWD prevalence is not spatially homogenous [47–50]. White-tailed deer are highly faithful to small home ranges in the Rocky Mountain West [11]. Prolonged prion shedding by CWD-positive does within their home range, including favored bedding locations, accompanied by communal grooming and shared home ranges with females provided opportunity for disease transmission through time.

Our study finding of higher incidence in does than bucks contradicts other reported studies that documented higher incidence in bucks than does (e.g., [45, 50, 51]). Presumably in hunted populations, bucks were the favored hunted sex as well. We believe that this discrepancy may be a function of the riparian habitat concentrating white-tailed deer and thus environmental contamination and allowing for the proposed role of does in the transmission of CWD in our study system. It is possible that in the future, when other habitats, such as winter lots in Wisconsin (where CWD has not been endemic for as long as Wyoming) have had similar time to become equivalently contaminated, does may become similarly important to transmission and incidence may increase in does in these population. In other words, perhaps our study population is an indicator of things to come, where initially bucks experience higher incidence until a threshold is met when does experience higher CWD incidence. This scenario assumes concentrated environmental contamination, however. For wide-ranging and dispersed populations, bucks may always experience higher incidence than females.

It is important to note that hunters may have had a bias in regards to harvesting collared deer. It is possible that hunters avoided shooting collared does in lieu of harvesting an uncollared doe to avoid altering the study results and to not have to deal with the hassle of returning a collar. Hunters targeting bucks may not have had such concerns if the antler size was large enough. If this was the case, then we may have over-emphasized the ratio of bucks to does in the harvest ratio. We believe this bias was relatively minor, at least within the main study site that encompassed the majority of the winter range, because hunters were forced to use one hunting outfitter on the VR Ranch and after conversations with this outfitter, they at least claimed to not be biased for or against harvesting collared animals.

Pregnancy and recruitment results indicate CWD does not compromise reproduction in female white-tailed deer. Blanchong et al. [52] also determined pre-clinical CWD did not negatively impact female reproduction in Wisconsin white-tailed deer. No difference in pregnancy indicates does participate in the rut regardless of CWD infection-status. It was not possible to determine if there was a difference in pregnancy and recruitment between pre-clinical and clinical CWD-positive does. However, it was common during the study to find one or two near-term fetuses in clinical-CWD female carcasses during the third trimester (Cornish and Edmunds, unpublished data). It is likely that fetuses exacerbate emaciation and hasten the death of does with terminal CWD. Our findings suggest does in pre-clinical disease give birth to fawns and are as successful at raising fawns to early September as CWD-negative does. Equal reproduction by CWD-positive does should dampen somewhat the negative effects of CWD on deer populations. Future research on neonate and young fawn survival is warranted, specifically to address the ability of CWD-positive white-tailed deer does to raise young to the age of population recruitment.

Pregnancy-specific protein B (PSPB) is not 100% accurate; Duquette et al. [53] documented 5 cases where white-tailed deer were found to be pregnant by trans-abdominal ultrasound but were deemed nonpregnant by PSPB. However, overall they found strong agreement between the two methods and recommended using either depending on the study objectives. We feel comfortable that the PSPB was an appropriate test, but it is possible that we underestimated pregnancy rates and therefore overestimated λ_1, which already is extremely low for a white-

tailed deer population. Considering the high pregnancy rates reported in this study, the impact on λ_1 from inaccurate tests likely was minimal.

The modeling exercise that determined λ_1 can be expected to be less than 1.0 (assuming other vital rates remain constant) at a prevalence of 27% suggests that as CWD in a population approaches these values, wildlife managers may choose to switch their objectives from lowering CWD prevalence by decreasing deer density to one of maintaining a sustainable population. The hunting-free Leslie matrix indicated removing additive hunting mortality in female deer resulted in a sustainable population. Therefore, it is recommended that at high CWD prevalence, hunting of does should be limited or ceased if the objective is to maintain population numbers. Currently this is a rare situation in most CWD endemic areas due to the relatively short period of time CWD has been present in most locations; this population should serve as an indication of what can happen at high prevalence when CWD has been endemic for an extended time period. Through time as prevalence rises in other endemic populations, more managers will be forced to make these choices if more effective management strategies or treatments are not developed. This recommendation is contingent on continued surveillance and monitoring of CWD in deer and elk populations in endemic areas as well as few or only minor public health concerns regarding CWD transmission to humans or livestock. Furthermore, if it becomes possible to accurately target and remove CWD-positive deer in a cost-effective manner, this management approach should be implemented in these populations where non-targeted culling is likely to be detrimental to population sustainability.

This population highlights the potential long-term negative outcome of endemic CWD to population sustainability and stresses the importance of preventing CWD from becoming endemic in a population, rather than attempting to manage it after the fact. Therefore, as previously suggested [43], the best management strategy remains minimizing movement of CWD to new areas.

Supporting Information

S1 Fig. Study Area Map. The study area, including Deer Creek drainage and VR Ranch southwest of Glenrock, Wyoming where deer were captured, tested for chronic wasting disease, marked with radio transmitters, and tracked by radio telemetry (2003–2010).
(TIF)

S1 Table. Causes of Mortality. Cause of mortality by sex and chronic wasting disease (CWD)-status of white-tailed deer captured, CWD-tested annually, radio-collared, and monitored by radio-telemetry on Deer Creek Drainage, southwest of Glenrock, WY (2003–2010). The χ^2 analysis determined if CWD-positive deer were over-represented in mortality data based on 23.8% average annual CWD-prevalence. Analysis was based on observed and expected total CWD-negative and CWD-positive deer. The CWD-unknown deer were excluded from analyses and sex was not considered.
(DOCX)

S2 Table. Leslie Matrix Population Model Sensitivity and Elasticity. Sensitivity and elasticity analysis of the 18 x 18 transition matrix, **A**, for the Leslie matrix population model for a chronic wasting disease (CWD)-endemic white-tailed deer population captured, CWD-tested annually, radio-collared, and monitored by radio-telemetry SW of Glenrock, WY (2003–2010). Results presented by age class-specific survival (CWD-negative (-) and CWD-positive (+)), fecundity, and CWD incidence sensitivity and elasticity results. Age class-specific survival, fecundity, and CWD incidence were incorporated into transition matrix, **A.**
(DOCX)

S3 Table. Pregnancy Data Used in Analyses.
(XLSX)

S4 Table. Recruitment Data Used in Analyses.
(XLSX)

S5 Table. Survival Data Used in Analyses.
(XLSX)

S6 Table. Incidence Data Used in Analyses.
(XLSX)

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Author Contributions

Conceived and designed the experiments: DRE FGL WEC TJK TEC.

Performed the experiments: DRE BAS FGL WEC TJK RGG TEC.

Analyzed the data: DRE MJK BAS.

Contributed reagents/materials/analysis tools: FGL WEC TJK TEC.

Wrote the paper: DRE MJK BAS FGL WEC TJK TEC.

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RESEARCH ARTICLE

Endemic chronic wasting disease causes mule deer population decline in Wyoming

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Abstract

Chronic wasting disease (CWD) is a fatal transmissible spongiform encephalopathy affecting white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and moose (*Alces alces shirasi)* in North America. In southeastern Wyoming average annual CWD prevalence in mule deer exceeds 20% and appears to contribute to regional population declines. We determined the effect of CWD on mule deer demography using age-specific, female-only, CWD transition matrix models to estimate the population growth rate (λ). Mule deer were captured from 2010–2014 in southern Converse County Wyoming, USA. Captured adult ($\geq$ 1.5 years old) deer were tested ante-mortem for CWD using tonsil biopsies and monitored using radio telemetry. Mean annual survival rates of CWD-negative and CWD-positive deer were 0.76 and 0.32, respectively. Pregnancy and fawn recruitment were not observed to be influenced by CWD. We estimated $\lambda = 0.79$, indicating an annual population decline of 21% under current CWD prevalence levels. A model derived from the demography of only CWD-negative individuals yielded; $\lambda = 1.00$, indicating a stable population if CWD were absent. These findings support CWD as a significant contributor to mule deer population decline. Chronic wasting disease is difficult or impossible to eradicate with current tools, given significant environmental contamination, and at present our best recommendation for control of this disease is to minimize spread to new areas and naïve cervid populations.

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Introduction

Chronic wasting disease (CWD) is a fatal transmissible spongiform encephalopathy affecting white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and moose (*Alces alces shirasi)* in North America [1–5]. All transmissible spongiform encephalopathies are caused by unconventional infectious agents composed of the proteinase-resistant pathologic isoform (PrP^{res}) of the normal cellular prion protein (PrP^{C}) [6–8]. Chronic wasting disease naturally occurs in free-ranging cervid populations in 21 U.S. states and two Canadian provinces [9], but limited information exists regarding the population-level impacts of CWD in the wild. In captivity, annual CWD incidence may exceed 50% in mule deer and white-tailed deer [10] and epidemics often end in the depopulation of deer at research facilities. Declines in free-ranging mule deer in Table Mesa, Colorado, elk in Rocky Mountain National Park in Colorado, and white-tailed deer in southeastern Wyoming were attributed to CWD prevalence greater than 13% [11–13]. From 2001–2009, the Wyoming Game and Fish Department (WGFD) recorded an average CWD prevalence of 31% from hunter harvested mule deer in southern Converse County, Wyoming [14]. Concurrently, WGFD estimated a >50% reduction in the South Converse Mule Deer Herd [SCMDH; 14]. High annual CWD prevalence and declining population trends in this mule deer herd warranted investigation of the influence of CWD-associated declines in vital rates (i.e. survival, pregnancy, and fawn recruitment) and population growth rate (λ).

We hypothesized that CWD negatively impacted adult survival, pregnancy, and recruitment of fawns. The effect of CWD on population growth was measured by estimating λ for the CWD test-postive and test-negative portions of the population. Prior research revealed mule deer possessing at least one phenylalanine (F) at codon 225 of the prion protein gene (*Prnp*) were less susceptible to CWD infection compared to homozygous serine (S) genotyped deer [15]. Therefore, we evaluated the influence of *Prnp* on CWD incidence and compared λ estimates of the phenylalanine genotype (225SF and 225FF deer grouped and hereafter referred to as 225^{*}F deer) and homozygous serine genotype (225SS) segments of the population [15]. Other studies suggested CWD-positive deer are more likely to be killed by mountain lions (*Puma concolor*) [16] and that mountain lions may selectively prey on prion-infected deer [17]. Mule deer also may be more vulnerable to vehicle collisions, especially during the later stages of infection [18]. Sympatric CWD-positive white-tailed deer were more likely to be harvested by hunters [13]. Thus, we evaluated if CWD-positive deer were more susceptible to specific causes of mortality.

Material and methods

Study area and population

We studied mule deer from the SCMDH that wintered primarily within the LaPrele Valley that surrounds the LaPrele Reservoir in southern Converse County, Wyoming from 2010–2014. The aggregate home range of all marked mule deer occupied an area ~2,576 km^2. Deer wintered at elevations of ~1,500 m and a portion of the population migrated to summer ranges at ~2,700 m. Our study area was predominantly comprised of private native rangelands with some cultivated meadows along with some small tracts of public land. Some mule deer seasonally migrated to higher elevations where larger tracts of national forest occurs. True mountain mahogany (*Cercocarpus montanus*), antelope bitterbrush (*Purshia tridentata*), and big sagebrush (*Artemisia tridentata*) dominated the foothills while sagebrush and irrigated hayfields dominated the lowland areas. In 2010, the WGFD estimated the SCMDH at ~6,100 deer and by the conclusion of the study in 2014, the herd was estimated at ~5,100 deer based on post-

harvest population estimates and different modeling techniques for 2010 (POP-II, Fossil Creek Software) and 2011–2014 (spreadsheet model) [19]. The hunting of does and fawns was largely eliminated in 2009 in response to poor population performance [14,19]. Throughout the course of this study, a seven-day general antlered mule deer season occurred in this population with approximately 300 males harvested each year within the herd unit [19]. Annual CWD prevalence of sympatric male and female white-tailed deer and elk harvested during the study averaged 13.32% (n = 42) and 5.92% (n = 529), respectively.

Captures and field data collection

We aerial net-gunned adult mule deer during winter (February/March) [20], focusing primarily on females and capturing at least 40 females each year. Males were captured from 2011–2014 to evaluate sex-associated CWD prevalence and survival. Captured deer were chemically immobilized with an intramuscular (IM) injection of either 0.03 mg/kg of carfentanil and 0.7 mg/kg of xylazine or 0.5 mg/kg of butorphanol, 0.35 mg/kg of azaperone, and 0.22 mg/kg of medetomidine (BAM; [21,22]). We collected blood by jugular venipuncture and used it for *Prnp* determination using restriction fragment length polymorphism with confirmation by sequencing of PCR fragments of random samples [15]. Serum separated from blood samples was used for pregnancy analysis by pregnancy-specific protein B (PSPB) concentration (Bio-Tracking LLC, Moscow, Idaho, USA). Approximate age at capture was determined using tooth eruption and wear [23]. Incisors from recovered carcasses were aged using cementum annuli analysis [24]. We assessed body condition by assigning a subjective score based on palpation of fat and muscle. Tonsil biopsies were performed to test deer for CWD by immunohistochemistry (IHC) and surgical equipment was cleaned using methods previously published to prevent iatrogenic transmission [25]. Deer were administered subcutaneous procaine/benzathine penicillin G combination (25,000 units/kg based on benzathine fraction, Bimeda, Le Sueur, Minnesota, USA) and 1.5 mg/kg of Banamine IM (Intervet Inc., Merck Animal Health, Summit, New Jersey, USA). Deer were given naltrexone (100 mg/mg of carfentanil) and tolazoline (2mg/kg) or naltrexone (50–100 mg), tolazoline (200–300 mg), and atipamezole (15–25 mg) to reverse anesthetic effects of carfentanil/xylazine or BAM, respectively [21,22]. Deer were fitted with either a store-onboard global positioning system (GPS) radio-collar (Lotek Wireless Inc., Newmarket, Ontario, Canada) or a very-high-frequency (VHF) radio-collar (Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA) equipped with mortality signal that was activated after 4 hours of inactivity. Deer were tagged with a large cattle ear-tag with an identification number and contact information if harvested for postmortem CWD testing and a metal WGFD identification ear-tag. Surviving deer were recaptured annually and processed as described with the exception of known CWD-positive deer, which no longer required biopsy. The study was completed in 2014 with the removal of radio-collars and final release of all surviving deer. All procedures involving deer were performed under the approval of the University of Wyoming Institutional Animal Care and Use Committee (No. A-3216-01) and the Wyoming Game and Fish Department (Permit No. 33–751).

Radio-collared mule deer were monitored at least twice weekly and mortalities were recovered to determine cause of death. Mortalities were investigated immediately after detection to recover carcasses prior to scavenging and autolysis. Necropsies were performed either in the field or at the Wyoming State Veterinary Laboratory. Postmortem CWD tests were performed when feasible. Retropharyngeal lymph nodes (RLNs) were collected and one was tested for PrP^{res} using the enzyme-linked immunosorbent assay (ELISA; [26]). Tonsil, obex region of the medulla oblongata, and the other RLN were tested for PrP^{res} by IHC [27]. We determined cause of death as clinical CWD if a deer tested positive for CWD postmortem and presented

with no other signs of disease and trauma. While CWD is a fatal disease, not all CWD-positive deer died due to clinical disease. The proximate cause of death was recorded for each mortality case regardless of CWD status at the time of necropsy.

Fawn recruitment (number of fawns per marked doe) was documented during November ground surveys from 2011–2013. Females that were pregnant during captures were located via telemetry. If fawns were not seen with marked females during the initial observation, females were displaced to observe fawns fleeing the area. If no fawns were observed during the first attempt, subsequent attempts were made until the end of November.

Kaplan-Meier survival and incidence analysis

Annual survival and incidence were estimated using Kaplan-Meier known-fates estimation [28,29]. Survival was estimated from previous capture event (t-1) to current capture event (t) and all deer entered the study analysis at $t = 0$ regardless of initial capture year. Survival and incidence were determined based on biological year (June 1st–May 31st), which formed the basis of stage-structured Lefkovitch matrix models [30]. Daily survival and weekly CWD incidence were calculated using the *survival* package v.2.37–7 [31] and *survfit* function in statistical program R v.3.1.0 [32]. Mortality dates were determined as the first mortality event recorded by the GPS radio-collar following initial capture. Mortality date for deer tagged with VHF radio-collars was determined either by the condition of the carcass or as the midpoint date between a live observation and a dead observation. Deer were right censored if they were lost to follow-up due to relocation failure, died from unnatural causes such as poaching or capture-related mortality, or survived to the end of the study. Several deer started the study as CWD-negative and subsequently tested positive, thus their survival time was split into two datasets, in which they were right censored as a CWD-negative deer at their CWD-positive test date. Survival estimates of CWD-positive deer used in our analyses were potentially biased low because deer were considered CWD-positive the day they tested positive and we included deer that were initially captured as test-positive animals. Post hoc survival analysis revealed that our survival estimate that included all CWD-positive deer fell within the 95% CI (0.28, 0.69) of 26 deer that experienced a CWD incident event during the study (we left censored 17 deer without known fates because they were test-positive post-mortem or test-positive during the last capture when monitoring ceased). Survival was determined separately based on sex, CWD status, age-class, and *Prnp* genotype. Deer were left censored when calculating incidence if they were initially CWD-positive. An incident event occurred when a CWD-negative deer first tested CWD-positive and right censored if lost to follow-up, CWD status was not determined on subsequent captures, or study ended with a final CWD-negative test. Incidence was calculated separately based on sex, age-class, and *Prnp* genotype. Log-rank tests [29,33] were performed in R using the function *survdiff* to compare all Kaplan-Meier curves [31].

Extended Cox proportional hazards model analysis

We examined the effects of sex, age at capture, CWD status, and *Prnp* genotype on weekly survival probability. An extended Cox proportional hazards model was used to determine which variables had the most influence on annual survival of deer [29,34]. The analyses were performed using the *coxph* function in R [31]. Time-dependent variables were created for CWD status and age as both changed through time for individual deer during the study [29]. Proportional hazards assumption was tested using the *cox.zph* function, which evaluates correlation between the Schoenfeld residuals and survival time [31]. Covariates failed proportionality when their p-value ≤ 0.05 [29]. Stepwise forward and backward selection of models were performed using the function *stepAIC* in the package *MASS* v.7.3–31 [35]. Models were ranked

based on Akaike's Information Criteria (AIC) values [36]. Model AIC values within 2 AICs of the best model (Δ AIC) were considered good predictors of survival and individual covariate p-values were evaluated for final model selection [36].

Pregnancy and recruitment mixed model analysis

We used generalized linear mixed models to determine the effects of age, CWD status, winter body condition, *Prnp* genotype, and observation year on annual proportion pregnant deer and fawn recruitment. A repeated measures analysis was performed and data grouped by unique deer identification was modeled using the *glmer* function in program *lme4* v.1.1–7 [37]. Pregnancy and recruitment indices were calculated separately based on CWD status and observation year.

Population growth rate estimation

An age- and CWD-structured, female-only Lefkovitch matrix model was used to estimate λ in MATLAB® (The MathWorks, Inc., Natick, MA, USA; [38]. We used a pre-breeding census, in which deer were counted prior to the birth-pulse in June, thus the first age-class in our model was yearling. Our matrix (Fig 1) represented both CWD-negative and CWD-positive deer of age x_i, where a deer could survive to age $x_i + 1$ at a probability of $\hat{\theta}_{i-}(1-\hat{\rho}_i)$, where $\hat{\theta}_{i-}$ was the probability of a CWD-negative deer surviving and $(1-\hat{\rho}_i)$ was the transition probability of remaining CWD-negative. Deer that were CWD-negative survived and became CWD-positive at a probability of $\hat{\theta}_{i-}^{\frac{1}{2}}\hat{\theta}_{i+}^{\frac{1}{2}}(\hat{\rho}_i)$, which represents continuous disease transmission from time t to t+1, and CWD-positive deer survived at a probability of $\hat{\theta}_{i+}$.

The vital rates included in the matrix model were pregnancy ($\hat{\beta}$), fawn recruitment ($\hat{\delta}$), overwinter fawn survival ($\hat{\theta}_0$), adult survival ($\geq$ 1 year old) of CWD-negative deer ($\hat{\theta}_-$), adult survival of CWD-positive deer ($\hat{\theta}_+$), and CWD incidence ($\hat{\rho}$). Fawns were not captured in our study; therefore, overwinter fawn survival from mid-December to mid-June was estimated from comparable areas in Colorado, from 1997 to 2008 [39]. Due to small sample size and non-significant differences in survival and fecundity among $\geq$ two-year-olds, we built our matrix model to include two age-classes, yearlings and adults. We calculated the 95% confidence interval for λ using previously described methods of parametric bootstrapping [40,41],

$$\begin{bmatrix} 1(-) \\ 1(+) \\ 2(-) \\ 2(+) \end{bmatrix}_{n_{(t+1)}} = \begin{bmatrix} 0 & 0 & \hat{\beta}\hat{\theta}_0\hat{\delta}(0.5)(1-\hat{\rho}) & \hat{\beta}\hat{\theta}_0\hat{\delta}(0.5)(1-\hat{\rho}) \\ 0 & 0 & \hat{\beta}\hat{\theta}_0\hat{\delta}(0.5)(\hat{\rho}) & \hat{\beta}\hat{\theta}_0\hat{\delta}(0.5)(\hat{\rho}) \\ \hat{\theta}_-(1-\hat{\rho}) & 0 & \hat{\theta}_-(1-\hat{\rho}) & 0 \\ \hat{\theta}_-^{1/2}\hat{\theta}_+^{1/2}\hat{\rho} & \hat{\theta}_+ & \hat{\theta}_-^{1/2}\hat{\theta}_+^{1/2}\hat{\rho} & \hat{\theta}_+ \end{bmatrix}_{A(n_t)}$$

Fig 1. Lefkovitch matrix model A representing transition of a female-only, pre-breeding, chronic wasting disease-structured 4 x 4 matrix of a mule deer population in southern Converse County, WY using demographic and disease rates observed from 2010–2014. n_t represents the number of deer in each age class by CWD status (-; PrP^{CWD} not detected and +; PrP^{CWD} detected). $\hat{\theta}_{(-,+)}$ represents estimated survival of CWD-negative or CWD-positive deer and $\hat{\theta}_{(0)}$ is the estimated fawn survival from mid-December to mid-June. $\hat{\rho}$ represents CWD incidence, $\hat{\beta}$ is the estimated pregnancy rate, and $\hat{\delta}$ is the estimated recruitment rate determined in November.

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modified using our vital rates and matrix configuration. Overwinter fawn survival was bootstrapped using the standard deviation published for the point estimate [39]. Sensitivity and elasticity analyses were performed to evaluate how sensitive λ was to changes in individual vital rates using the method of Morris and Doak [41].

We initially ignored the influence of *Prnp*-genotype on CWD incidence and used the overall female-only CWD incidence during the biological year in our matrix. However, to understand the effect of CWD incidence on λ, we varied annual incidence in the matrix from 0% to 100% and calculated the change in λ. Additionally, we calculated λ using genotype-specific incidence rates to examine estimated growth rates for the 225SS and 225*F segments of the population. The approach used to model λ for all scenarios assumes constant vital rates, thus density dependence was not represented in model results.

Results

Annual CWD prevalence and incidence

During the study, 143 mule deer were captured (118 female, 25 male) and *Prnp* genotypic frequencies were 78% 225SS deer and 22% 225*F deer. Average annual CWD prevalence was 24% (95% CI = 22%–27%). Male CWD prevalence was higher throughout the study (average = 43%) compared to female CWD prevalence (average = 18%). Seventy-seven deer tested positive for CWD during the study, of which 43 were deer that transitioned from test negative to positive. Annual CWD incidence did not differ among observation years ($\chi^2 = 3.2$, df = 3, p = 0.36) and did not increase suggesting iatrogenic transmission likely did not occur. Also, annual CWD incidence was not different among age-classes ($\chi^2 = 8.5$, df = 7, p = 0.29) and between sex for years 2011 ($\chi^2 = 0.3$, df = 1, p = 0.56) and 2012 ($\chi^2 = 2.7$, df = 1, p = 0.10). In 2013, annual CWD incidence was significantly higher in males than females ($\chi^2 = 6.1$, df = 1, p = 0.01). Annual CWD incidence differed among genotypes ($\chi^2 = 34.5$, df = 2, $p < 0.01$), with 225SS deer more likely to become CWD-positive compared to 225*F deer. Average annual female CWD incidence was 0.26 (SE = 0.04) and genotype-specific incidence used in our matrix models were 0.49 (SE = 0.05) for 225SS deer and 0.02 (SE = 0.06) for 225*F deer.

Cause-specific mortality

We documented 97 mortalities of radio-collared deer. Mule deer that were CWD-positive were more susceptible to mountain lion predation (n = 20; $\chi^2 = 6.36$, df = 1, p = 0.01), hunter harvest (n = 4; $\chi^2 = 7.98$, df = 1, $p < 0.01$), and illegal harvest (n = 2; $\chi^2 = 3.99$, df = 1, p = 0.05). Mountain lion predation was the number one cause of mortality followed by clinical CWD (n = 14). Other natural causes of mortality of radio-collared mule deer included vehicle collision (n = 3), coyote predation (n = 1), fence entanglement (n = 1), drowning (n = 1), and winter kill (n = 1). Thirteen deer died due to injuries sustained during captures and we were unable to determine the cause of death in 37 cases due to severe autolysis and scavenging.

Extended Cox proportional hazards models

Stepwise selection based on AIC values of our extended Cox proportional hazards models resulted in four competing models. The model that incorporated sex and CWD was selected as the best model for predicting survival based on individual covariate p-values (Table 1). Male mule deer were twice as likely (Hazard Ratio = 2.08, p = 0.01, 95% Confidence Interval (CI) = 1.18–3.68) to experience a mortality event compared to females and CWD-positive deer were over three times more likely (Hazard Ratio = 3.30, $p < 0.0001$, 95% CI = 1.98–5.49) to die during our study compared to CWD-negative deer. Genotype was included in our models

Table 1. Extended Cox proportional hazards models with *a priori* variable selection of parameters that potentially influenced mule deer survival in southern Converse County, WY from 2010–2014.

Model	Model parameters	*K*	AIC	Δ AIC
1	Sex[a], CWD[b]	2	557.51	0
2	Sex, CWD, Age*t	3	557.78	0.27
3	Sex, CWD, Age*t, CWD*Age*t	4	557.98	0.47
4	Sex, CWD, Age*t, Sex*CWD	4	559.03	1.52
5	CWD, Age*t	2	561.18	3.67
6	Sex, Age*t	2	577.98	20.47

K, number of parameters; AIC, Akaike information criterion; ΔAIC, difference with best model AIC value; CWD, chronic wasting disease; t, time

Age*t, time-dependent covariate of age

*, interaction.

[a] Hazard Ratio = 2.08, 95% Lower Confidence Interval (LCI) = 1.18, 95% Upper Confidence Interval (UCI) = 3.68, $P = 0.01$

[b]Hazard Ratio = 3.30, 95% LCI = 1.98, 95% UCI = 5.49, $P < 0.01$

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initially; however, models did not converge because they lacked full representation of the *Prnp* genotypes in both CWD categories (CWD-negative and CWD-positive). Only one 225SF deer tested CWD-positive out of 29 deer, but was censored after 154 weeks and neither of the two 225FF deer captured tested CWD-positive during the study. Therefore, we removed *Prnp* genotype from model analysis and we were not able to determine the influence of genotype on CWD-positive survival probability.

Annual survival estimates

Kaplan-Meier annual survival was significantly different between CWD-negative and CWD-positive deer ($\chi^2 = 40.10$, df = 2, $p < 0.01$), CWD-negative and CWD-positive females ($\chi^2 = 38.30$, df = 2, $p < 0.01$), and CWD-negative females and CWD-negative males ($\chi^2 = 9.00$, df = 2, $p = 0.002$; Table 2). Estimated annual survival of CWD-negative deer (0.76, SE = 0.04) was considerably higher than CWD-positive deer (0.32, SE = 0.06; Table 2). Female deer survived at a rate of 0.79 (SE = 0.04) annually compared to 0.50 (SE = 0.16) annual survival of male deer; however, this sex-associated difference was not observed for CWD-positive deer (Table 2). When comparing female and male survival curves, both declined at similar rates until a dramatic decline in male survival around 250 days that corresponded to the short hunting season (Fig 2). This accelerated rate of decline in survival curves at about 250 days was prominent when comparing CWD-negative and CWD-positive males (Fig 2). A similar pattern of accelerated decline was observed between CWD-negative and CWD-positive females starting around day 275 (Fig 2).

Annual survival was not significantly different among age-classes for either CWD-negative ($\chi^2 = 7.00$, df = 5, p = 0.22) or CWD-positive deer ($\chi^2 = 0.80$, df = 4, p = 0.936). Therefore, when combining adult age-classes ($\geq$ 2 years old) for our matrix model and survival from June 1st–May 31st of CWD-negative females and CWD-positive females, survival was 0.85 (SE = 0.13) and 0.38 (SE = 0.34), respectively. Survival of CWD-negative females among genotypes was marginally significant ($\chi^2 = 5.8$, df = 2, p = 0.05) with higher survival of 225*F deer compared to 225SS deer.

Annual pregnancy and recruitment estimates

Mean annual pregnancy of CWD-negative and CWD-positive females was 0.99 (SD = 0.11, 95% CI = 0.97–1.00) and 0.94 (SD = 0.24, 95% CI = 0.88–1.00), respectively (Table 3). Fawn

Table 2. Kaplan-Meier survival rates and log-rank test results by sex, age, and chronic wasting disease (CWD) status of mule deer in southern Converse County, WY from 2010–2014.

Category	Results	Overall	1.5	2.5	3.5	4.5	5.5+
CWD (-) vs. (+) deer	**Survival: CWD (-)**	0.76	0.63	0.67	0.91	0.70	1.00
	Survival: CWD (+)	0.32	0.00	0.15	0.60	0.28	0.51
	χ^2	40.10	1.70	14.70	19.20	4.20	2.50
	P-value	0.00	0.19	0.00	0.00	0.04	0.12
CWD (-) vs. (+) females	**Survival: CWD (-)**	0.79	0.67	0.70	0.97	0.67	1.00
	Survival: CWD (+)	0.37	0.00	0.20	0.61	0.34	0.44
	χ^2	38.30	1.10	14.50	23.20	2.20	2.90
	P-value	0.00	0.31	0.00	0.00	0.14	0.09
CWD (-) vs. (+) males	**Survival: CWD (-)**	0.50	0.50	0.33	0.50	n = 1	n = 0
	Survival: CWD (+)	0.19	0.00	0.00	0.50	0	n = 1
	χ^2	1.10	0.20	0.00	0.10		
	P-value	0.29	0.70	0.83	0.78		
CWD (-) females vs. males	**Survival: females**	0.79	0.67	0.70	0.97	0.67	1.00
	Survival: males	0.50	0.50	0.33	0.50	n = 1	n = 0
	χ^2	9.00	0.20	1.60	2.40		
	P-value	0.00	0.62	0.21	0.12		
CWD (+) females vs. males	**Survival: females**	0.37	0.00	0.20	0.61	0.34	0.44
	Survival: males	0.19	0.00	0.00	0.50	0.00	n = 1
	χ^2	2.60	1.00	0.00	0.20	4.80	
	P-value	0.11	0.32	0.99	0.64	0.03	

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recruitment from birth to November was similar between CWD-negative (average = 0.48, SD = 0.65, 95% CI = 0.33–0.63) and CWD-positive deer (average = 0.56, SD = 0.65, 95% CI = 0.30–0.82; Table 4). Age, winter body condition, CWD status, *Prnp* genotype, and observation year did not influence pregnancy and recruitment of fawns ($p > 0.05$).

Population models

Our matrix model estimated $\lambda = 0.79$ (0.72, 0.87) that corresponded to a 21% annual decrease in the population with a population half-life of 4 years. The models that assumed 100% CWD prevalence and 0% CWD prevalence estimated $\lambda = 0.51$ and $\lambda = 1.00$, respectively (Fig 3). Using the estimated CWD incidence for 225SS deer, we estimated $\lambda = 0.64$ and for 225*F, $\lambda = 0.98$. The matrix model was most sensitive to changes in survival of CWD-negative deer ($\hat{\theta}\_$) and CWD incidence ($\hat{P}_i$; Table 5). However, when the sensitivities of vital rates were rescaled to account for proportional changes (elasticity), only changes in CWD-negative survival had largest effect on λ (Table 5).

Discussion

Our findings support CWD as a population-limiting disease of mule deer with the potential to cause dramatic declines that resemble local population extinction. Other studies have found a negative association between CWD prevalence and λ [11,12,40,42], but none have documented λ estimates resulting from endemic CWD as low as those reported here. The only scenario in which population growth rate was stable ($\lambda = 1$) was in the absence of CWD. Even without CWD mortality, we predicted that this population would not grow under current conditions. This finding was unremarkable considering mule deer populations throughout North America

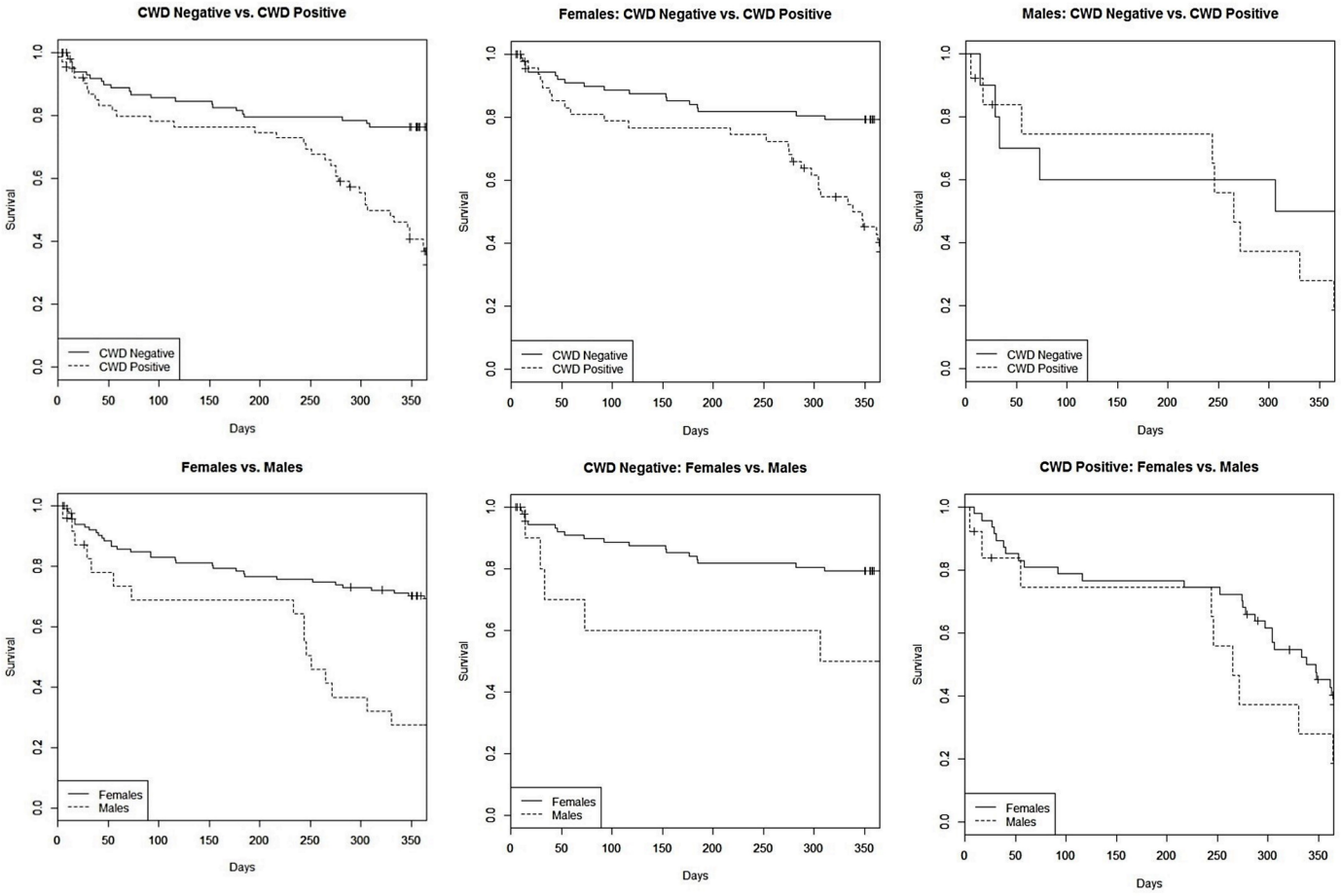

Fig 2. Kaplan-Meier annual survival curves of free-ranging mule deer in southern Converse County, Wyoming captured as part of a study investigating the population-level impacts of chronic wasting disease from 2010–2014.

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are underperforming in the absence of CWD [43]. Chronic wasting disease may exacerbate population declines in herds that are currently considered CWD-free. From 2010–2014, we predicted the southern Converse County herd would decline by >50% using our estimated λ = 0.79 and a starting population size of ~6,100 deer. Earlier models of CWD epidemics in mule deer using prevalence observed in our study herd forecasted similar dramatic outcomes

Table 3. Proportion of mule deer that were pregnant at approximately 75 days bred in southern Converse County, WY.

	CWD-Negative	CWD-Positive
Year	**Proportion pregnant (LCI,UCI)**	**Proportion pregnant (LCI, UCI)**
2010	1.00 (1.00, 1.00)	0.88 (0.64, 1.00)
2011	0.97 (0.91, 1.00)	0.91 (0.73, 1.00)
2012	1.00 (1.00, 1.00)	0.94 (0.83, 1.00)
2013	1.00 (1.00, 1.00)	0.95 (0.85, 1.00)
2014	0.95 (0.86, 1.00)	1.00 (1.00, 1.00)
Average	0.99 (0.97, 1.00)	0.94 (0.88, 1.00)

LCI, 95% lower confidence interval; UCI, 95% upper confidence interval.

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Table 4. Proportion of fawns at heel during November recruitment surveys of radio-collared female mule deer that were either CWD-test negative or positive during winter captures in southern Converse County, WY.

	CWD-Negative	CWD-Positive
Year	**Fawns/Doe (LCI,UCI)**	**Fawns/Doe (LCI, UCI)**
2011	0.48 (0.24, 0.72)	0.29 (0.00, 0.65)
2012	0.40 (0.17, 0.63)	0.56 (0.08, 1.03)
2013	0.56 (0.26, 0.86)	0.78 (0.34, 1.21)
Average	0.48 (0.33, 0.63)	0.56 (0.30, 0.82)

LCI, 95% lower confidence interval; UCI, 95% upper confidence interval.

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[44,45]. This population has experienced population declines of approximately 50% based on WGFD population estimates prior to the start of our study [19]. However, this population did not appear to decline as dramatically during the study as our estimate of λ would suggest based on WGFD population estimates (approximately a 4% decline from 2010 to 2014) [19]. While the 2010 and 2014 population size estimates were not strikingly different, the general trend over time suggests a declining population. From 2011 to 2012, WGFD estimated a 19% decline in mule deer numbers and a 15% decline the following year [19]. These declines observed during our study fall within our 95% CI for λ (0.72, 0.87). In 2013, greater spring precipitation ended a year-long drought and moderate winter conditions resulted in a 5% increase of the population estimate in 2014 [19]. Therefore, while the population experienced productive years and deer numbers increased; these increases were marginal compared to the larger declines observed over multiple years.

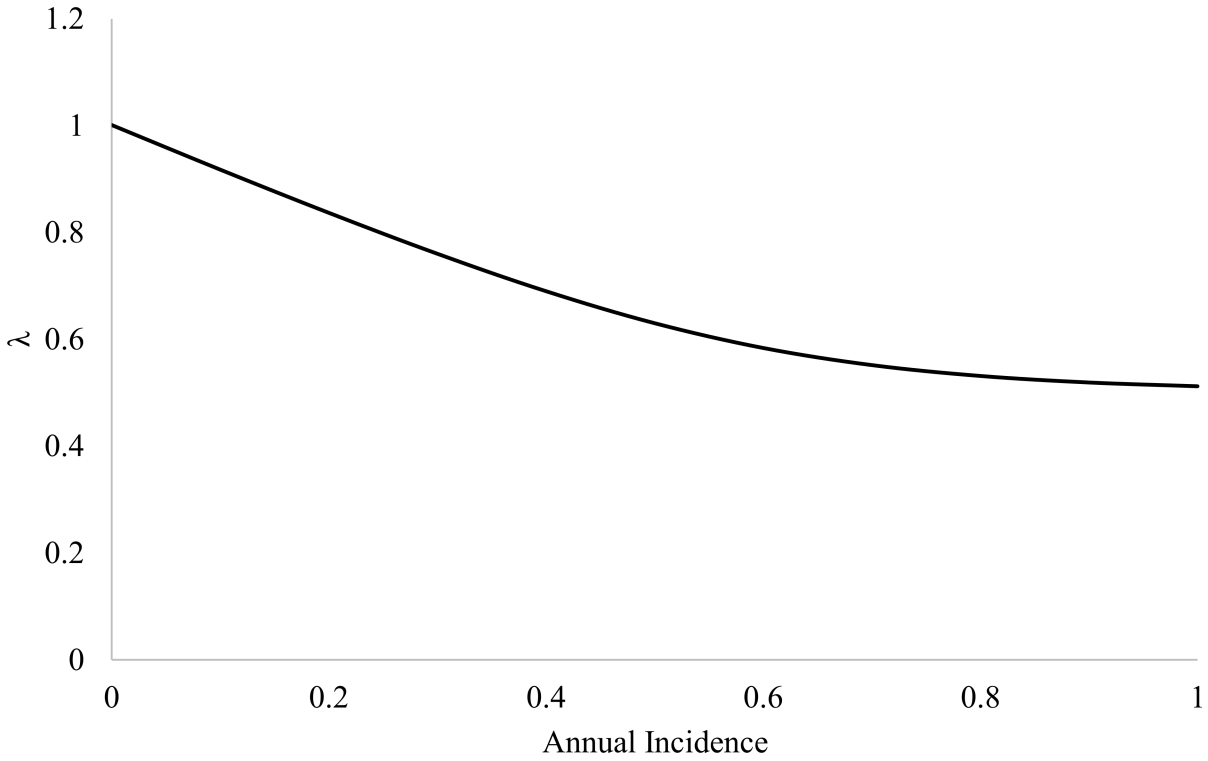

Fig 3. Chronic wasting disease (CWD) annual incidence and its effect on finite rate of population growth (λ; solid line) when all other vital rates were kept constant in our Lefkovitch matrix model of a mule deer population in southern Converse County, WY (2010–2014).

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Table 5. Sensitivities and elasticities of vital rates included in our Lefkovitch matrix model representing a mule deer population in southern Converse County, WY from 2010–2014.

Vital rate	Symbol	Estimated value	Sensitivity	Elasticity
Pregnancy rate	$\hat{\beta}$	0.97	0.1461	0.1753
November recruitment	$\hat{\delta}$	0.51	0.2779	0.1753
Over-winter survival rate of fawns	$\hat{\theta}_0$	0.72	0.1968	0.1753
Survival rate of CWD negative deer	$\hat{\theta}_-$	0.85	0.7126	0.7491
Survival rate of CWD positive deer	$\hat{\theta}_+$	0.38	0.1609	0.0756
CWD incidence rate	$\hat{\rho}_i$	0.26	-0.6939	-0.2231

https://doi.org/10.1371/journal.pone.0186512.t005

We did not find disease-associated declines in reproduction for mule deer, nor have they been observed in sympatric white-tailed deer [40]. Females were pregnant regardless of CWD status during captures when they were approximately 75 days bred. Despite evidence that suggests CWD-positive mule deer recruit fewer fawns than CWD-negative deer [11], we did not detect a difference in fawn recruitment based on CWD status. Even with a reduction in fawn recruitment of CWD-positive mule deer in Colorado, inclusion of this vital rate in models did not significantly influence λ [11]. While CWD did not have a detectable impact on annual pregnancy and recruitment, lifetime reproduction of prime-aged females was likely reduced due to increased annual mortality of CWD-infected individuals. Prion-infected Table Mesa mule deer in Colorado survived an additional 1.6 years on average compared to 5.2 years for uninfected deer [16]. Furthermore, fawns produced by CWD-negative deer, which more likely possessed the more resistant genotype compared to CWD-positive deer in our study, potentially contributed to the increase of the F allele in the population.

Prion protein genotype was important in determining CWD infection and influenced λ for *Prnp*-specific segments of the population. As was expected, mule deer that possessed the 225SS *Prnp* genotype were more likely to be CWD-positive compared to 225SF and 225FF deer in our study. We only detected one 225SF CWD-positive deer even though 225*F deer comprised 22% of the study population. Two radio-collared 225FF deer were captured in 2013 and survived to study termination in 2014 with negative tonsil biopsy IHC results. However, evidence suggests current IHC techniques may have lower sensitivity in detecting CWD-positive tissues of 225FF mule deer [46]. Both 225FF deer were estimated to be 3.5 years old during their initial capture, both were pregnant in 2013 and 2014, and during 2013 recruitment surveys, one had a single fawn at heel. The other 225FF deer was not observed during 2013 fawn recruitment surveys. During 2014 captures, ultrasound revealed that one 225FF deer was pregnant with twins and the other was pregnant with a single fetus. Based on a small sample size, free-ranging 225FF mule deer appeared to be as ecologically fit as 225SS deer. The few 225FF mule deer observed in captivity were characterized as atypical in behavior, body condition, and reproductive performance [46]. Formal investigations looking at the effects of *Prnp* genotype on fitness are necessary to determine how populations with greater numbers of 225FF mule deer will persist despite their reduced susceptibility to CWD.

Estimates of λ for 225SS and 225*F segments of the population were mediated by varying CWD incidence rates. Using 225SS CWD incidence in our matrix model, we estimated an annual population decline of 33% of 225SS deer. A model incorporating 225*F CWD incidence estimated an annual population decline of 1%. These results suggest the 225*F segment of the population was nearly stable while the 225SS segment of the population was declining rapidly. Using previously published data of mule deer genotyped in the early 2000s from the

same geographic area [15], we estimated a 10% population increase in the F allele frequency in less than 10 years [47]. Other factors were not identified that may potentially increase F allele frequency in the absence of CWD as it was outside of the scope of our study. Adaptation to CWD has previously been demonstrated in elk [48] and white-tailed deer [49] using empirical data and statistical models.

Natural selection in favor of less susceptible *Prnp* genotypes may be assisted with selective predation by mountain lions and harvest by hunters of prion-infected deer. While CWD-positive deer were more likely to be killed by mountain lions compared to uninfected deer, it is not clear if this source of mortality regulated or influenced the observed CWD epidemic. Selective predation of CWD-positive deer in Table Mesa, Colorado did not appear to control CWD transmission [16] and it also did not appear to curtail CWD prevalence in the current study herd. Theoretic modeling incorporating 15% predation rate and four times greater risk of predation of prion-infected deer resulted in the eradication of CWD in a closed population [50]. While we observed one year of 15% predation of marked deer in 2010, 3–4% predation rate was typical for most years of the study and it never exceeded 15%. While direct mortality could decrease the subset of infected animals in a population, predators may also act as mechanical vectors that spread prions across the landscape. Infectious prions were demonstrated to pass through the digestive system of coyotes (*Canis latrans*) three days post ingestion suggesting the potential role of carnivores in prion transport and spread [51]. At this time, empirical evidence that supports a predator influence on CWD epidemics does not exist. However, with the expected spread of CWD into areas such as the Greater Yellowstone Area that is occupied by several large predators (i.e. wolves (*Canis lupus*), grizzly bears (*Ursus arctos*), and mountain lions), the role of predators in prion transmission dynamics may soon become more relevant [50]. A multi-predator system may have a greater impact on an emerging CWD epidemic, especially before significant prion contamination occurs in the environment.

Hunting mortality was minimal in our study, although it appeared that sympatric CWD-positive mule deer and white-tailed deer were selectively harvested [13]. It is unclear why others have found no difference in hunting risk between infected and uninfected deer [52], but it is logical that CWD-positive individuals are more vulnerable to harvest due to behavioral changes associated with the disease. The precipitous decline in survival of CWD-positive males increased predictably during the short hunting season around day 250. However, unpredictably there was an observed accelerated decline in the survival curve of CWD-positive females after day 275. Multiple factors may have contributed to greater mortality of CWD-positive females on winter range including increased risk of predation and stressors associated with the rut, hunting season, recruitment of fawns, and winter conditions. Regardless of the cause, CWD-positive deer were more likely to die on winter ranges. This has important implications for the spread and translocation of CWD across the landscape. Congregating deer on winter range may act as a source for CWD-infection to disparate populations when deer migrate in the spring to different summer ranges. These temporal behaviors could explain some of the spatial heterogeneity of CWD prevalence across the landscape [53].

Without an effective CWD vaccine or treatment, management of this disease is limited to focusing on those individuals that are not yet prion-infected. According to our sensitivity analysis, changes in CWD-negative adult survival would cause the greatest changes in λ. Improving survival of uninfected mule deer may partially mitigate the impact of CWD. However, to achieve close to stable population growth rates required an unrealistic scenario of 100% survival of CWD-negative deer under high CWD prevalence conditions. We observed low fawn recruitment (0.51 fawns) during the study regardless of disease status compared to an adjacent herd (0.68 fawns) located north of SCMDH [54] and populations throughout the species range ($>$ 0.75 fawns) [11,55]. Management strategies that focus on improving both adult survival of

CWD-negative deer and fawn recruitment may increase λ. Mule deer populations that currently experience low adult and fawn survival should be closely monitored for CWD because our models predicted less than ideal outcomes once CWD was established.

Lastly, we predicted stable population growth only when CWD prevalence was reduced to 0% in our model. Eradication of CWD is an improbable goal in endemic areas, especially where CWD has been detected for over a decade and potentially present for over 50 years [45]. However, these findings highlight the importance of preventing or slowing the spread of CWD to naïve populations. Mule deer populations currently undergoing declines in the absence of CWD, such as in Nevada and South-central British Columbia [56,57] and in western Wyoming, should be routinely surveyed for detection of CWD. Intensive surveillance that could detect the first few positive CWD cases and rapid removal of prion-infected individuals may be the difference between an established epidemic and local CWD eradication as apparently accomplished in New York and Minnesota [58,59]. While most state agencies focus efforts on collecting hunter harvested and road-killed deer for CWD testing, we recommend incorporating predator-killed deer to the repertoire based on our finding of greater susceptibility of CWD-positive deer to predation [16,17]. Many other non-disease-associated factors contribute to declining mule deer populations and CWD could be the fatal consequence for many herds. Due to the lack of effective management tools to eliminate CWD once established, we suggest management focus efforts and research on how to slow or potentially prevent the movement of CWD across the landscape into uninfected populations.

Conclusions

With this study, we have demonstrated the long-term consequences of endemic CWD on a free-ranging mule deer population. Chronic wasting disease caused significant declines in the study mule deer herd as well as in sympatric white-tailed deer [13]. Unlike sympatric white-tailed deer, where removal of female harvest may permit λ to increase to stable levels based on model estimates [13], elimination of the mule deer doe/fawn hunting season prior to the onset of our study did not result in $\lambda \geq 1$. A limited antlered-only harvest in this herd provides a reliable source for monitoring short-term CWD prevalence trends [53]. Additionally, improving and conserving critical mule deer habitats may diminish the negative impacts of CWD, but will not completely mitigate the undesirable population effect of CWD based on our model outcomes. Lastly, without the use of effective vaccines, treatments, and sustainable techniques to reduce CWD incidence, management can currently only focus on slowing the spread of CWD to CWD-free populations.

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Longitudinal Detection of Prion Shedding in Saliva and Urine by Chronic Wasting Disease-Infected Deer by Real-Time Quaking-Induced Conversion

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ABSTRACT

Chronic wasting disease (CWD) is an emergent, rapidly spreading prion disease of cervids. Shedding of infectious prions in saliva and urine is thought to be an important factor in CWD transmission. To help to elucidate this issue, we applied an *in vitro* amplification assay to determine the onset, duration, and magnitude of prion shedding in longitudinally collected saliva and urine samples from CWD-exposed white-tailed deer. We detected prion shedding as early as 3 months after CWD exposure and sustained shedding throughout the disease course. We estimated that the 50% lethal dose (LD_{50}) for cervidized transgenic mice would be contained in 1 ml of infected deer saliva or 10 ml of urine. Given the average course of infection and daily production of these body fluids, an infected deer would shed thousands of prion infectious doses over the course of CWD infection. The direct and indirect environmental impacts of this magnitude of prion shedding on cervid and noncervid species are surely significant.

IMPORTANCE

Chronic wasting disease (CWD) is an emerging and uniformly fatal prion disease affecting free-ranging deer and elk and is now recognized in 22 U.S. states and 2 Canadian provinces. It is unique among prion diseases in that it is transmitted naturally through wild populations. A major hypothesis to explain CWD's florid spread is that prions are shed in excreta and transmitted via direct or indirect environmental contact. Here we use a rapid *in vitro* assay to show that infectious doses of CWD prions are in fact shed throughout the multiyear disease course in deer. This finding is an important advance in assessing the risks posed by shed CWD prions to animals as well as humans.

Chronic wasting disease (CWD) is an emergent transmissible spongiform encephalopathy affecting free-ranging populations of mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and moose (*Alces alces*) in North America (1, 2). CWD is the only known prion disease to spread horizontally through wild populations, in which it continues to expand in prevalence and range in North America (3). As a prion disease, CWD is caused by a pathogenic, misfolded conformation of the normal, natively folded cellular protein PrP^{C} to a pathogenic prion conformer (variously designated PrP^{CWD}, PrP^{SC}, or PrP^{D}) (2, 4–7).

A leading hypothesis for the facile spread of CWD in wild populations is that the accumulation and excretion of CWD prions in bodily fluids facilitate both direct animal-to-animal transfer and substantial environmental contamination leading to indirect infection (8–10). Infectious CWD prions have been identified in urine, saliva, blood, and feces by bioassay of deer or cervid PrP^{C}-expressing transgenic mice (11–16). Prions bound to soil are remarkably stable, retaining infectivity even after a decade (9, 17–20). Moreover, some evidence suggests that prions bound to soil may increase infectivity through an unknown mechanism (21). Understanding the kinetics and magnitude of CWD prion shedding into the environment and assessing the risks to humans and other species remain significant yet unmet challenges.

Recent advances in the detection of prions at minute quantities and in diverse biological fluids, such as saliva, urine, and blood, allow for a thorough analysis of the shedding of CWD prions during the disease course (11, 14, 22, 23). In the present study, we used a rapid *in vitro* real-time prion protein conversion assay (real-time quaking-induced conversion [RT-QuIC]) (24) and an unprecedented number of longitudinal saliva and urine samples from white-tailed deer exposed to CWD prions by various routes (aerosol, oral, and environmental) to track the kinetics and magnitude of prion seeding activity and to estimate accrued prion shedding over the course of infection.

MATERIALS AND METHODS

Sourcing and inoculation of white-tailed deer. The longitudinal shedding kinetics of CWD in excreta were analyzed in three experimentally exposed cohorts of CWD-naive, hand-raised, indoor-adapted white-tailed deer ($n = 22$). Our long-time collaborators at the Warnell School of Forestry and Natural Resources, University of Georgia, provided CWD-free white-tailed deer fawns that were housed in the indoor CWD research facility at Colorado State University. All appropriate institutional protocols for animal handling and treatment were properly followed. Inocula-

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TABLE 1 Summary of IHC and RT-QuIC results for orally inoculated CWD-exposed deer[a]

Parameter	Value or description									
Animal no.	PO-1	PO2	PO-3	PO-4	PO-5	PO-6	PO-7	PO-8	PO-9	PO-10
Sex	M	M	M	M	F	M	F	M	F	F
Genotype	G/S	G/S	G/G	G/S	G/G	G/G	G/S	G/G	G/G	G/G
Time to positive biopsy specimen (mo p.i.)										
Tonsil	6	9	9	9	9	6	6	9	6	6
RAMALT	6	15	9	6	6	6	6	6	6	6
No. of positive specimens/total no. of specimens tested										
3 mo p.i.										
Saliva	0/8	0/8	0/8	0/8	0/8	0/8	1/8	0/8	0/8	0/8
Urine	0/8	NA	NA	0/8	NA	NA	0/8	NA	0/8	0/8
6 mo p.i.										
Saliva	0/8	0/8	NA	0/8	0/8	0/8	1/8	0/8	0/8	1/8
Urine	0/8	0/8	**5/8**	0/8	1/8	0/8	**2/8**	NA	0/8	1/8
9 mo p.i.										
Saliva	NA	0/8	**2/8**	NA	0/8	**8/8**	**3/8**	**8/8**	0/8	**4/8**
Urine	**4/8**	NA	NA	1/8	0/8	1/8	NA	NA	0/8	0/8
10 mo p.i.										
Saliva	0/8	1/8	1/8	**4/8**	NA	NA	**4/8**	**5/8**	NA	NA
Urine	1/8	NA	**3/8**	**4/8**	NA	0/8	NA	NA	NA	0/8
12 mo p.i.										
Saliva	0/8	0/8	**5/8**	**3/8**	0/8	1/8	0/8	**5/8**	1/8	**3/8**
Urine	**7/8**	NA	**2/8**	NA	0/8	NA	NA	NA	0/8	0/8

[a] A total of 22 indoor-adapted white-tailed deer were inoculated with CWD^+ brain homogenate via either aerosol, oral, or environmental exposure, the latter of which was done with feed, water, and bedding harvested from a separate suite containing CWD-infected deer. Data on aerosol- and environmentally exposed deer are shown in Tables 2 and 3. The sex of the deer and the genotype at PrP position 96 (G/G or G/S) are noted. Animals were monitored for CWD throughout the disease course by IHC of tonsil and RAMALT biopsy specimens. All aerosol and orally inoculated deer were PrP^{CWD} positive by tonsil biopsy between 6 and 9 months postinoculation. For the aerosol-inoculated deer, saliva and urine were collected at 3-month intervals during the subclinical phase of disease and more frequently in the clinical phase of the disease (i.e., after 15 months). Saliva and urine RT-QuIC results are reported as numbers of positive replicates among the total number of replicates, representing a minimum of 2 experiments. Results for positive samples are shown in bold. M, male; F, female; NA, not available.

tion methods and protocols to prevent cross contamination among study cohorts have been described previously (15, 25, 26). In short, aerosol-exposed deer received two 1.0-ml doses of a 5% CWD^+ brain homogenate via aerosolization (deer A-1 to A-6), the *per os* (p.o.)-exposed deer received a single, 1-g dose of CWD^+ brain orally (deer PO-1 to PO-10), and the environmental group was exposed to fomites from feed buckets, bedding, and water from CWD^+ deer suites every day for 19 months (E-1 and E-2), without any direct contact of the two animal groups. Sham-inoculated deer were exposed to negative brain homogenates by the aerosol or oral route and were housed in separate suites in the same building.

Sample collection. Body fluids and excreta (saliva, blood, urine, and feces) were collected along with tonsil and recto-anal mucosa-associated lymphoid tissue (RAMALT). Biopsy specimens were serially collected from all exposed deer cohorts at intervals of 3 months or less from the study start to termination (up to 2 years) (Tables 1 to 3). Due to sample tissue or body fluid availability, however, not all samples were collectable at each time point (Tables 1 to 3). All animals were monitored for CWD infection by immunohistochemistry (IHC) of tonsil and RAMALT biopsy specimens at each collection interval, as well as by clinical disease scoring (Table 4). At study termination, all deer were necropsied and multiple tissues collected for an array of assays.

Preparation of RT-QuIC reagents and recombinant SH-rPrP(90-231). Syrian hamster recombinant PrP containing amino acids 90 to 231 [SH-rPrP(90-231)] was prepared as described previously (14, 27). In summary, protein expression in 1-liter cultures was induced using Over Night Express (EMD-Millipore) autoinduction medium, and inclusion bodies were harvested by using Lysonase (EMD-Millipore) according to the manufacturer's protocol. Inclusion bodies were solubilized in 8.0 M guanidine hydrochloride (GuHCl) with 100 mM $NaPO_4$ for 1 h with rotation at room temperature. The solubilized rPrP was bound to superflow Ni resin (Qiagen) and refolded on the Ni column by using a 180-ml linear gradient from 6.0 M GuHCl, 100 mM $NaPO_4$, 10 mM Tris, pH 8.0, to the same buffer without the GuHCl, flowing at 0.75 ml/min. rPrP was eluted with a linear gradient from 100 mM $NaPO_4$, 10 mM Tris, pH 8.0, to 0.5 M imidazole in 100 mM $NaPO_4$, 10 mM Tris, pH 5.5, at 2.0 ml/min. The eluted protein was dialyzed in two changes of 4.0 liters of 20 mM $NaPO_4$ at pH 5.5. The concentration of SH-rPrP(90-231) was determined by measuring the A_{280}, and the protein was stored at 4°C.

RT-QuIC assay conditions. RT-QuIC reaction mixtures contained 20 mM $NaPO_4$, 1 mM EDTA, 320 mM NaCl, 0.1 mg/ml SH-rPrP(90-231), and 10 μM thioflavin T (ThT; Sigma). Shaking and reading settings were as previously reported (14). RT-QuIC reactions were deemed positive when the ThT fluorescence value reached a level beyond 5 standard deviations from the initial fluorescence value.

Preparation of samples for RT-QuIC. Saliva was thawed at room temperature and vortexed, and then 100 μl of undiluted saliva was transferred for further concentration of CWD prions as previously reported (14). A 4% solution of freshly prepared phosphotungstic acid (PTA; Sigma) was added to 100 μl saliva, to a final concentration of 0.3%. Samples were incubated for 60 min at 37°C, with shaking at 1,700 rpm, and were then centrifuged at 17,000 × *g* for 30 min. PTA-precipitated pellets were resuspended in 10 μl 1× phosphate-buffered saline (PBS) (20 mM $NaPO_4$, 150 mM NaCl, pH 7.4) with 0.1% sodium dodecyl sulfate (SDS). Two microliters of each sample was added in quadruplicate to a prepared RT-QuIC reaction mixture.

Urine samples were thawed at room temperature and vortexed, and

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TABLE 2 Summary of IHC and RT-QuIC results for aerosol-inoculated CWD-exposed deer[a]

Parameter	Value or description					
Animal no.	A-1	A-2	A-3	A-4	A-5	A-6
Sex	M	F	M	F	M	M
Genotype	G/G	G/G	G/G	G/G	G/G	G/G
Time to positive biopsy specimen (mo p.i.)						
Tonsil	6	9	6	9	6	6
RAMALT	12	6	6	9	6	6
No. of positive specimens/total no. of specimens tested						
3 mo p.i.						
Saliva	NA	NA	NA	NA	**7/8**	0/8
Urine	NA	0/8	0/8	NA	0/8	NA
6 mo p.i.						
Saliva	0/8	**2/8**	0/8	NA	**4/8**	NA
Urine	0/8	NA	NA	NA	**3/8**	NA
9 mo p.i.						
Saliva	NA	**8/8**	NA	**2/8**	NA	1/8
Urine	**5/8**	NA	NA	NA	**5/8**	NA
12 mo p.i.						
Saliva	0/8	**8/8**	0/8	NA	**2/8**	1/8
Urine	**2/8**	NA	0/8	NA	**8/8**	0/8
15 mo p.i.						
Saliva	1/8	**8/8**	**4/8**	NA	1/8	**6/8**
Urine	0/8	0/8	0/8	NA	0/8	0/8
16 mo p.i.						
Saliva	0/8	**4/8**	**3/8**	1/8	**5/8**	**6/8**
Urine	0/8	NA	0/8	NA	**4/8**	NA
19 mo p.i.						
Saliva	0/8	**4/8**	**8/8**	0/8	NA	NA
Urine	0/8	0/8	0/8	1/8	NA	0/8
20 mo p.i.						
Saliva	0/8	**8/8**	NA	**8/8**	0/8	†
Urine	1/8	1/8	0/8	NA	**6/8**	†
21 mo p.i.						
Saliva	0/8	**3/8**	**5/8**	**4/8**	**7/8**	†
Urine	0/8	**3/8**	0/8	**2/8**	NA	†
22 mo p.i.						
Saliva	NA	0/8	0/8	**2/8**	†	†
Urine	0/8	1/8	0/8	0/8	†	†
23 mo p.i.						
Saliva	0/8	0/8	0/8	**3/8**	†	†
Urine	0/8	1/8	0/8	0/8	†	†
25 mo p.i.						
Saliva	0/8	†	†	**3/8**	†	†
Urine	**2/8**	†	†	0/8	†	†
26 mo p.i.						
Saliva	**8/8**	†	†	†	†	†
Urine	NA	†	†	†	†	†

[a] See the footnote to Table 1 for further details. †, deer that died during the study.

TABLE 3 Summary of IHC and RT-QuIC results for environmentally exposed CWD-exposed deer[a]

Parameter	Value or description	
Animal no.	E-1	E-2
Sex	M	M
Genotype	G/S	G/G
No. of positive specimens/total no. of specimens tested		
Saliva		
0 mo p.i.	0/12	0/12
3 mo p.i.	1/12	**3/12**
6 mo p.i.	**2/12**	**6/12**
12 mo p.i.	**2/12**	**12/12**
15 mo p.i.	**2/12**	**9/12**
Urine		
6 mo p.i.	0/8	1/8
12 mo p.i.	0/8	0/8
15 mo p.i.	**2/8**	**8/8**
18 mo p.i.	0/8	**3/8**

[a] See the footnote to Table 1 for further details.

500 μl was transferred to a fresh tube and then centrifuged for 30 min at 17,000 × *g*. Supernatants were removed, and cell pellets were resuspended in 100 μl of 1× PBS. Seven microliters of a freshly prepared 4% solution of sodium phosphotungstic acid (NaPTA; Sigma) was added to the 100-μl suspension, to a final concentration of 0.3%. Samples were incubated for 60 min at 37°C, with shaking at 1,700 rpm, and then centrifuged at 17,000 × *g* for 30 min. Supernatants were removed, and cell pellets were resuspended in 16 μl of 0.05% SDS. Four microliters of each sample was added in quadruplicate to a prepared RT-QuIC reaction mixture.

Immunohistochemistry. Tissues from biopsy and necropsy specimen collections were fixed in paraformaldehyde-lysine-periodate (PLP) for 1 to 3 days and then transferred to 60% ethanol for long-term storage. Sections of obex, retropharyngeal lymph node, and tonsil were routinely processed and embedded in paraffin, and 5-μm sections were placed on positively charged slides. Slides were processed for PrP^{CWD} detection as previously described (28). Briefly, deparaffinized and dehydrated tissue sections were treated with 88% formic acid for 30 min prior to hydrated autoclaving antigen retrieval in a citrate buffer. The antigen signal was detected with an anti-prion antibody (F99/97.6.1) at a concentration of 10 μg/ml followed by an alkaline phosphatase-conjugated anti-mouse secondary antibody and was visualized with an alkaline phosphatase red kit, using an automated stainer (Ventana Medical Systems). Positive- and negative-control slides containing obex and retropharyngeal lymph node sections were run in parallel.

Calculation of infectivity. To determine the infectivity of excreted samples, the threshold for positivity was set as the average baseline fluorescence plus 5 standard deviations. Only samples with more than 6 positive results among the total of 8 replicates were analyzed. The threshold cycle (C_T) value was calculated for each sample by determining the time at which the reaction crossed the threshold. The amyloid formation rate could then be defined as the inverse of the C_T ($1/C_T$). Additionally, a standard curve was developed from the amyloid formation rates from an endpoint-bioassayed brain sample from a CWD^+ animal and fit to a log-linear line of best fit [$y = \mathrm{mlog}(x) + b$; calculated for 3 experiments with 4 replicates in each experiment] (27). The amyloid formation rates from the saliva and urine samples were interpolated on the standard curve to estimate the infectivity of the sample relative to the bioassayed reference brain homogenate. With the line of best fit, amyloid formation rates of saliva and urine samples were used to calculate the micrograms of CWD brain equivalents. The latter were translated to 50% lethal dose (LD_{50}) values by being divided by the LD_{50} of the reference bioassayed brain homogenate (27). Saliva and urine amyloid formation rates were calculated based on at least 2 experiments with at least 4 replicates each.

RESULTS

Kinetics of CWD prion shedding in saliva and urine. To better understand the onset and persistence of prion shedding over time, we analyzed longitudinally collected saliva and urine samples from deer exposed to CWD by aerosolization of CWD (deer A-1 to A-6), p.o. administration (deer PO-1 to PO-10), and environ-

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TABLE 4 Clinical disease stage scoring[a]

Time of scoring (mo p.i.)	Score for indicated animal															
	Orally inoculated group										Aerosol-inoculated group					
	PO-1	PO-2	PO-3	PO-4	PO-5	PO-6	PO-7	PO-8	PO-9	PO-10	A-1	A-2	A-3	A-4	A-5	A-6
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Termination of study	3	3	3	3	0	2	2	3	3	2						
16											0	0	0	0	0	0
19											1	1	1	1	2	3
20											2	1	2	1	2	†
21											2	2	2	1	†	†
22											2	2	2	1	†	†
23											2	3	3	2	†	†
25											2	†	†	3	†	†
26											3	†	†	†	†	†

[a] Deer were given a score of 0 to 4 for clinical disease at each time point when excreta samples were taken. 0, normal behavior; 1, subtle behavioral changes (diurnal rhythms and patterns of sleeping, feeding, and activity are altered); 2, mild but observable neurological deficits, commonly mild ataxia in the hindquarters; 3, early stage, behavioral changes continue, with early signs of deterioration and continued progression of ataxia; 3, late stage, gait abnormalities become pronounced; 4, neurological deficit progression, wide-legged stance, low-hanging head, piloerection, obvious signs of muscle wasting, and increased ataxia. The appetite and ability to eat and drink are intact, with dramatic increases often seen (2 to 3 times normal volumes). †, deer that died during the study.

mental fomite contact (E-1 and E-2). Each saliva ($n = 94$) and urine ($n = 65$) sample was analyzed in at least two experiments, with four replicates for each experiment. Blinded analysis of saliva and urine from CWD-negative animals resulted in a test specificity of 97.2% for saliva ($n = 104$ replicates) and 99.3% for urine ($n = 280$ replicates). No individual saliva or urine samples from CWD-naive deer had more than one false-positive replicate in two experiments with eight total replicates. Therefore, any saliva or urine sample analyzed in at least two experiments with more than one positive replicate was considered positive. Positive RT-QuIC wells were deemed positive when the thioflavin T fluorescence reached a value that was 5 standard deviations higher than the initial fluorescence value. Prion seeding activity was observed in saliva and urine from all infected deer at points throughout the long disease course, although we found considerable variation in detectable prion shedding among longitudinal sampling dates (Fig. 1 and Tables 1 to 3). Prion seeding activity in saliva was detected as early as 3 months postinoculation (p.i.) in an aerosol-exposed deer (Fig. 1, deer A5). Seeding activity in urine was detected at 6 months p.i. and later in the disease course (>12 months p.i.) (Fig. 1 and Tables 1 to 3). In all aerosol- and orally exposed deer, CWD prion seeding activities were relatively similar in terminal brain samples, indicating relatively similar endpoints of disease (Fig. 2).

Frequency of prion seeding activity in saliva and urine of deer exposed by mucosal routes. The temporal nature of prion shedding in CWD pathogenesis is pertinent to understanding the spread of the disease in cervids by direct and indirect environmental contact. Prion seeding activity was detected in ~50% of saliva samples collected from deer exposed by the aerosol or oral route (Fig. 3). Overall, there were significantly more positive test replicates for saliva samples from the aerosol-inoculated deer than for those from the orally inoculated deer ($P = 0.0067$), suggesting that higher prion loads are shed in saliva from deer exposed to CWD by that route. Urinary amyloid seeding activity was detected in ~25% of all samples tested in both the aerosol and oral exposure groups (24% for the p.o. group and 27% for the aerosol group). Four of the orally exposed deer had the 96G/S PRNP genotype, which was previously linked to a longer survival period than that for animals with the more frequent 96G/G genotype (30). However, the percentages of positive saliva and urine samples between 96G/S and 96G/G deer were not statistically different ($P = 0.20$ for urine and $P = 0.23$ for saliva) (Tables 1 to 3). All deer in the aerosol and oral exposure groups were IHC positive by 9 months p.i., by either tonsil or RAMALT biopsy. However, heterogeneity is often seen in biopsy specimens from live animals due to sampling difficulties and the availability of lymphatic tissues after multiple biopsy specimen samples have been taken (Tables 1 to 3).

Prion shedding in environmentally exposed deer. Two deer were exposed to CWD by a somewhat more natural route, i.e., transfer of used bedding, water, and feed from separate suites containing CWD-infected deer, with no direct contact between the two animal groups (26). Both environmentally exposed deer (E-1 and E-2) developed CWD infection, although both the time of detection and prion seeding loads at termination varied between them (Fig. 4). PrP^{CWD} and RT-QuIC seeding activity were readily detected in the terminal brain (obex region of the medulla), retropharyngeal lymph node, and tonsil from animal E-2 (Fig. 4). In contrast, in deer E-1, PrP^{CWD} was detected only in three tonsil lymphoid follicles at terminal collection, and low levels of prion seeding activity were detected in the tonsil, retropharyngeal lymph node, and brain (Fig. 4). Additionally, deer E-2 showed slightly lower amyloid formation rates in terminal obex/brain stem samples than those of the aerosol-exposed group of deer, and as shown above, deer E-1 showed only marginal seeding activity in the terminal obex/brain stem (Fig. 5). Thus, deer E-1, which had the 96 G/S genotype, was likely at an earlier stage of infection progression (Table 3 and Fig. 5). Prion amyloid seeding

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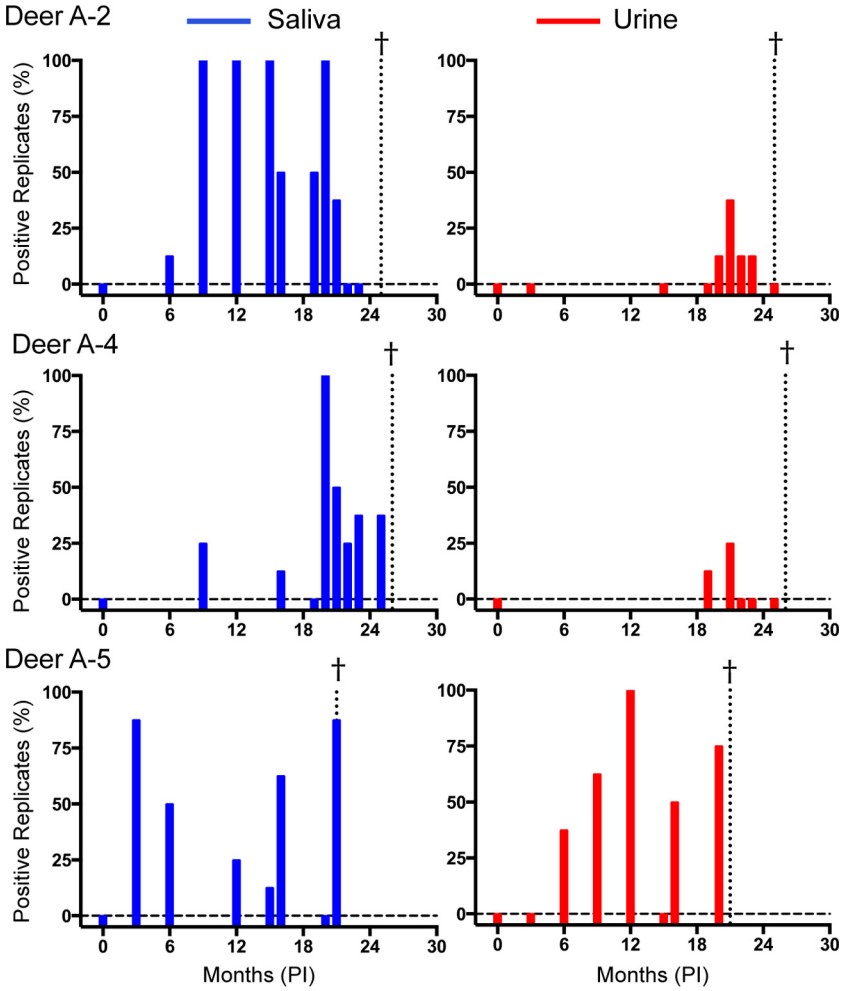

FIG 1 Shedding of CWD prions in aerosol-exposed deer. Saliva results are shown in blue (left), and urine results are shown in red (right). RT-QuIC results for saliva and urine are from two experiments with eight total replicates. The data for three deer of six from the aerosol-inoculated group are shown to illustrate the trends observed. Each sample tested is represented by a bar, with between 0 and 100% replicates testing positive. Negative samples are expressed as a bar that meets the zero line (dashed horizontal line); samples that were not available have no bar. The dotted vertical line marked with a cross represents the terminal sample for the indicated animal.

activity was detected in saliva and urine from both of the environmentally exposed deer, beginning as early as 3 months in deer E-2, which, interestingly, had the most consistent prion shedding observed in this study despite having less seeding activity in terminal brain tissue (Fig. 6A and B and Table 3).

Estimation of infectivity in saliva and urine samples. To better characterize the magnitude of prion shedding, we applied a previously described approach based on relating the amyloid formation rate to a bioassayed reference brain homogenate to estimate the relative level of lethality (LD_{50}) in excreta samples (27). Amyloid formation rates assayed by RT-QuIC were expressed as 1/time for ThT fluorescence emission to cross the threshold (C_T). Thus, a higher amyloid formation rate ($1/C_T$) indicates a greater concentration of amyloid seeds, analogous to the results of quantitative real-time PCR, wherein lower C_T values indicate higher initial concentrations of DNA seeds (27, 31). We determined that the amyloid formation rates of saliva and urine samples producing ≥6 positive results among 8 replicates (Fig. 7A) were equivalent to the rates produced by 10^{-6} to 10^{-7} dilutions of a reference (10% [wt/vol]) CWD^+ brain homogenate (Fig. 7A).

To help substantiate this approach, we also analyzed the amyloid formation rates of two historical saliva samples (from deer 133 and 144) that had previously been bioassayed in cervidized transgenic mice (13, 14, 32) (Fig. 7A). The rates for each of these previously bioassayed saliva samples were similar to the rates found in the present longitudinal study (Fig. 7A). Samples of 300 μl of saliva from deer 133 and 144 produced 500-day attack rates of ~50% (14). When the same samples were analyzed by quantitative RT-QuIC, the extrapolated LD_{50}s for saliva of deer 133 and 144 were estimated to be 494 ± 202 μl and 411 ± 168 μl, respectively, thus resembling the volume of saliva producing ~1 LD_{50} in cervidized mice.

The LD_{50} values for saliva and urine samples collected at time points of more than or close to 1 year p.i. exhibited higher amyloid formation rates, implying that higher concentrations of prion infectivity are shed later in disease progression (Fig. 7B and Tables 1 to 3). While the volume of saliva shed is surely much smaller than that of urine, the level of extrapolated infectivity was ~10-fold greater than that in urine (i.e., ~1.0 versus 0.1 transgenic mouse LD_{50}/ml in urine). Nevertheless, given that the average volume of

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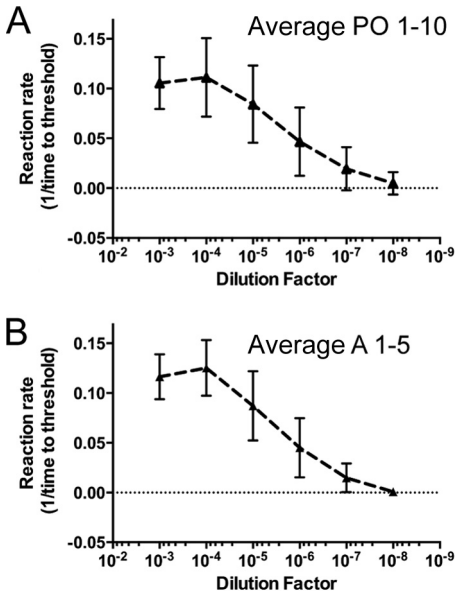

FIG 2 Similar levels of CWD seeding activity were observed in terminal obex samples from orally and aerosol-inoculated deer. The average amyloid formation rate ($1/C_T$) was plotted for the orally inoculated group (A) and the aerosol-inoculated group (B). The reaction rate ($1/C_T$) was determined by dividing by the time in hours until an RT-QuIC reaction crossed the experimental threshold (5 standard deviations [SD] from the initial fluorescence value) (*y* axis). Larger numbers signify higher amyloid formation rates. The dilution factor represents a series of 10-fold dilutions of a 10% homogenate of a terminal obex/brain stem sample (*x* axis). Error bars represent 1 SD for all averaged rates for all animals in each inoculation group. Amyloid formation rates were calculated for at least two experiments and at least four replicates (per experiment) of each serial dilution from 10^{-2} to 10^{-8} for each brain sample.

urine excreted daily by a 100-kg deer is ~1 liter, a CWD-infected deer would deposit an estimated 100 (cervidized mouse) LD_{50} daily into the environment (33).

DISCUSSION

The geographic region in which CWD has been detected has continued to expand in the last decade (http://www.nwhc.usgs.gov). While the factors that influence CWD spread remain incompletely understood, direct and indirect/environmental exposure to shed prions remains the leading hypothesis. To better understand the magnitude and mechanisms of CWD spread, we analyzed the longitudinal shedding of prions in saliva and urine of white-tailed deer exposed to CWD by mucosal exposure routes. We documented prion shedding as early as 3 months postexposure and estimated that an infected deer would excrete thousands of prion infectious doses over its disease course. We also found little difference in prion shedding between deer of the more susceptible 96G/G and more resistant 96G/S genotypes (30) (although our sample size was small). However, after CWD infection was established in G/S deer, they displayed shedding kinetics and levels similar to those of G/G deer. Perhaps due to selective pressures imposed by CWD in nature, G/S deer are more prominently represented in older age classes (34). In theory, a slower disease progression combined with a larger population fraction could lead to a larger environmental contamination impact attributable to CWD-infected G/S deer (34).

Interestingly, we observed that persistent environmental exposure to presumed low levels of excreted CWD prions was associated with prominent prion seeding activity detected in the saliva and urine of a deer so exposed (Fig. 4 to 6 and Tables 1 to 3). Perhaps exposure to repeated low prion doses in nature may in

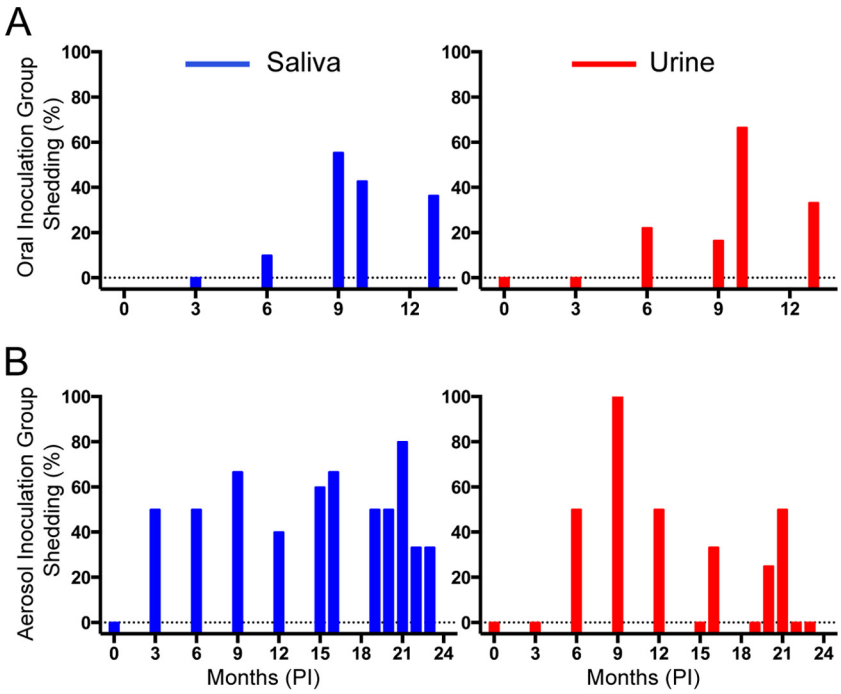

FIG 3 Frequencies of prion shedding in CWD-exposed deer. Saliva and urine data are shown in blue and red, respectively. (A) Percentages of total positive samples in which shedding was detected for the orally inoculated deer (≥2 of 8 replicates were positive). (B) Percentages of samples wherein shedding was detected for the aerosol-inoculated deer (≥2 of 8 replicates were positive). Bars that meet zero (horizontal dotted line) are time points where no shedding was detected, and samples that were not available have no bar.

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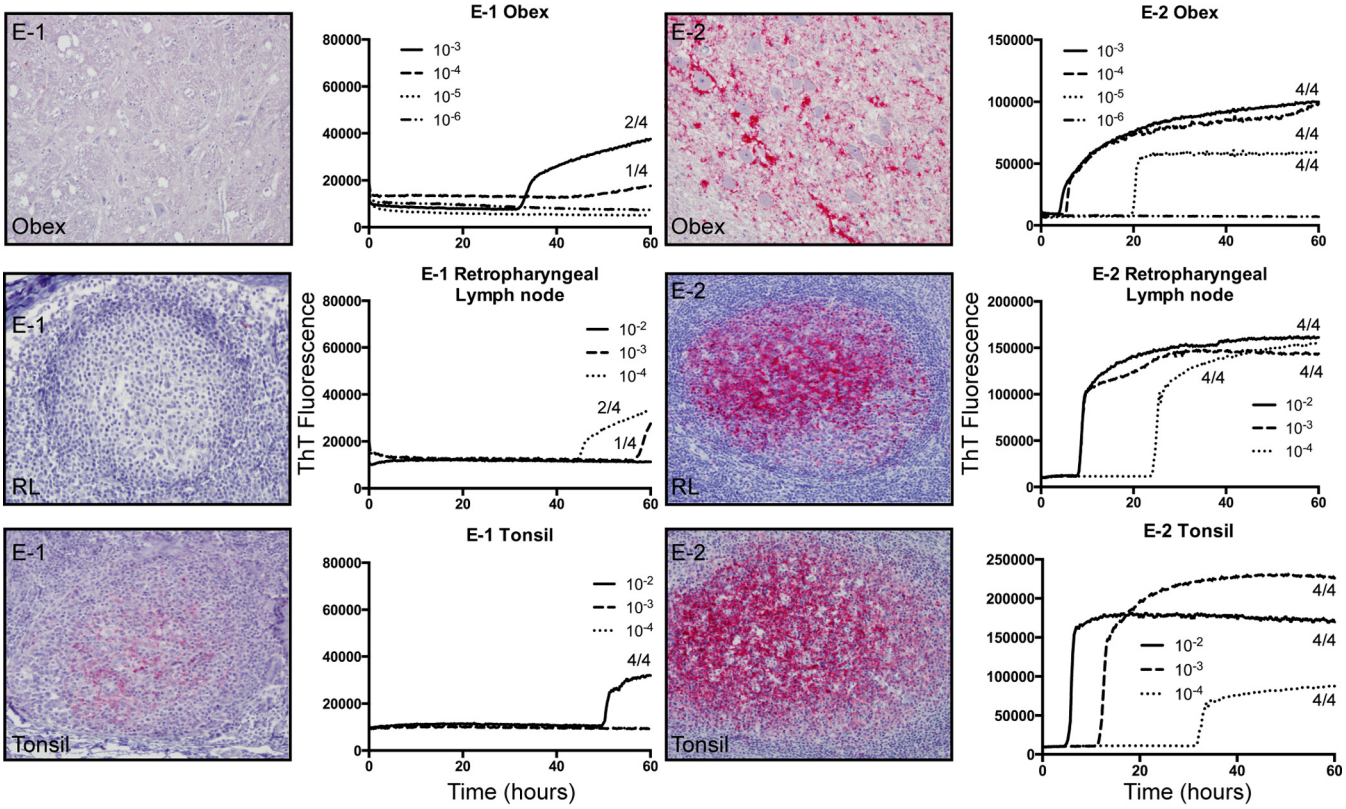

FIG 4 Analysis of environmentally exposed animal terminal disease state. IHC results and representative RT-QuIC data for deer E-1 and E-2 are displayed. Results are for one replicate each for serial dilutions of obex (10^{-3} to 10^{-6}), retropharyngeal lymph node (10^{-2} to 10^{-4}), and tonsil (10^{-2} to 10^{-4}) samples. Each serial dilution was repeated in two experiments, with four replicates in each experiment. The number of total positive replicates for each dilution is noted. IHC staining for PrP^{CWD} is characterized by red granular deposits in the neuropil of the obex and in germinal centers of lymphoid follicles in the tonsil and retropharyngeal lymph node. IHC staining was performed with the antibody F99/97.6.1. Magnification, ×20.

turn lead to more consistent prion shedding by animals so infected, as has previously been inferred for subinfectious doses of scrapie (35), although substantially more data would be needed to support this extrapolation. Additional information is needed to assess the infectivity of excreta deposited in the environment; however, prion shedding of this magnitude in a free-ranging species would seem to pose a substantial challenge to eradication of CWD. The risk to humans and other species posed by florid dissemination of CWD prions into the environment, while unclear, cannot be discounted in light of more recent evidence that barriers to cross-species infection may not be absolute (36–39).

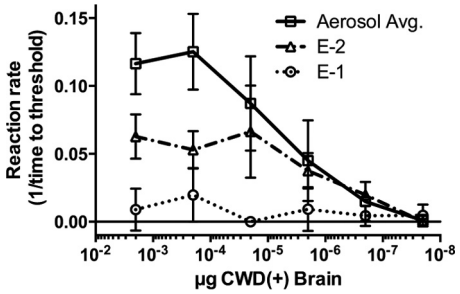

FIG 5 Quantitation of obex sample data for environmentally exposed deer. Amyloid formation rates in serially diluted obex samples from deer E-1 (dotted line) and E-2 (dashed-dotted line) and an average amyloid formation rate for the entire CWD^+ aerosol-inoculated group (solid line) are compared. The amyloid formation rate was calculated as previously described. Larger numbers signify higher amyloid formation rates. Error bars represent 1 standard deviation from the mean.

We have made significant progress in detection of prion amyloid seeding activity in excreta, saliva, and blood (11, 14, 27); however, assay inhibitors of these complex biological materials may yet remain. Thus, our quantitative estimation of prion shedding may be understated (Fig. 1 to 4 and Tables 1 to 3). Moreover, due to the presence of inhibitors, our sampling of excreta was restricted to small volumes (100 μl for saliva and 500 μl for urine) compared to what is actually shed in the environment. It seems likely that temporal gaps in our detection of CWD prions in excreta reflect limits in our ability to detect seeding activity rather than natural oscillations in prion shedding. Thus, we continue to explore more practical and effective means of enrichment and/or enhancement to better address the needle-in-the-haystack aspect of prion detection in excreted and environmental samples.

Prion shedding from mucosal surfaces is not limited to CWD. Prion seeding activity has been detected in body fluids or excreta of scrapie-infected sheep and hamsters (40–44), as well as bovine spongiform encephalopathy (BSE)-infected cattle (45). Amyloid seeding activity has also been detected in cerebrospinal fluid of human patients with sporadic Creutzfeldt-Jakob disease (sCJD)

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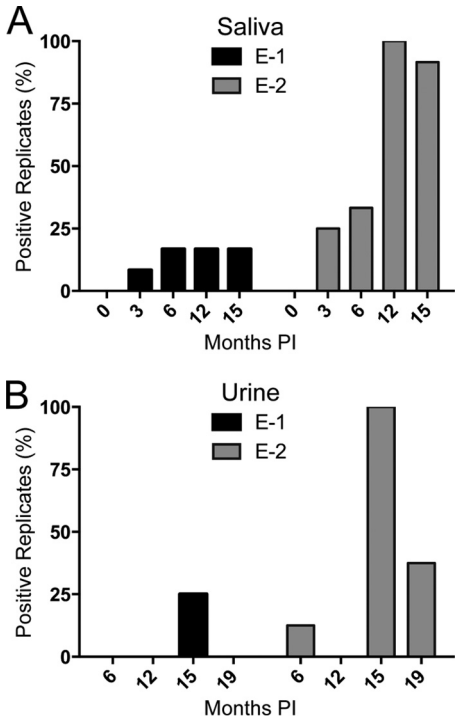

FIG 6 Longitudinal analysis of salivary and urinary shedding in environmentally exposed deer. (A) For saliva analysis, the percentage of positive replicates among a total of 12 replicates representing 3 independent experiments was plotted for each time point. (B) Urine RT-QuIC results for 2 experiments with 4 replicates each, with 8 replicates total for each time point.

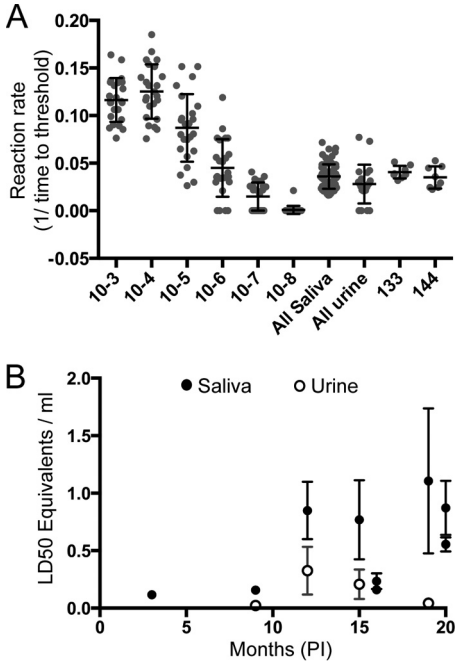

FIG 7 Comparison of amyloid formation rates and LD_{50} equivalents in saliva and urine during the CWD disease course. (A) Amyloid formation rates were plotted for 10-fold dilution series of terminal obex samples and saliva and urine samples from deer in this study where at least 6 of 8 total replicates were positive and for bioassayed saliva samples from deer 133 and 144. (B) The Tg(cerPrP)mouse LD_{50} was calculated for 1 ml of either saliva or urine and plotted over time of disease. Data for saliva samples are shown by filled circles, and those for urine samples are shown by open circles. Error bars represent 1 standard error of the mean.

(46), highlighting the potential mucosal egress of prions in humans with sCJD.

While directly comparable assay methods have not been applied to the study of sheep scrapie, our estimations of shed infectivity in saliva and urine in CWD appear to be consistent with the studies of Maddison et al. (40), Gough et al. (47), and others, using serial protein misfolding cyclic amplification (sPMCA) and bioassays. Again, estimating the infectious prion loads deposited in the environment is complicated by both the potential intermittent nature of shedding and uncertainties about the stability of prion infectivity in environmental niches and surfaces (9, 20). Nevertheless, the importance of environmental contamination in CWD is supported by the studies of Miller et al. (18, 48), wherein naive deer repopulating pastures that previously housed prion-infected deer also became infected. Evidence that soil-bound prions retain infectivity has been supplied by the studies of Seidel et al. (49).

The species barrier limiting transmission of CWD prions to humans appears to be substantial (50, 51), as no case of human prion disease has yet been linked to CWD (52, 53). However, works by Castilla et al. (38), Barria et al. (36, 39), Cassard et al. (37), and others show that the species barrier may be more dynamic than previously estimated. It remains unknown whether natural passage of excreted CWD prions through generations of outbred cervids in nature may ultimately alter its species/transmission barrier. Thus, a more complete understanding of the transmission, excretion, environmental contamination, and species barrier for this emergent prion disease is warranted.

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Chanel Tewalt

From: Dr. Scott Leibsle
Sent: Thursday, June 9, 2022 4:07 PM
To: Lloyd Knight
Cc: Chanel Tewalt
Subject: Fwd: Rulemaking - IDAPA - Domestic Cervidae

Please add to the Cervidae rule making record.

Scott R. Leibsle DVM, DABVP (Eq)
State Veterinarian/Administrator - Animal Industries
Idaho State Department of Agriculture

Begin forwarded message:

> **From:** Rulon Jones <utahelkhunt@gmail.com>
> **Date:** June 9, 2022 at 3:44:09 PM MDT
> **To:** "Dr. Scott Leibsle" <Scott.Leibsle@isda.idaho.gov>
> **Subject: Rulemaking - IDAPA - Domestic Cervidae**
>
> All across the country, when CWD is found in an area we have seen a big variety of reactions. Some states have done very little, Colorado for one. They have been known to have CWD longer than any other State. What has been the result of CWD in free ranging cervidae in Colorado? No change in free ranging populations since identifying CWD there! Colorado currently has the largest free ranging elk and mule deer population of any state in the country. Doing very little has worked for them. Our neighboring state of Wyoming has had the same approach with a similar result. They also were one of the first to recognize CWD presence. There has been no impact on total numbers of wild populations in Wyoming.
> Some of the more drastic reactions from state agencies to CWD have been similar to what we saw with the Fish and Game here in Idaho. This approach is to be much more proactive, kill lots of animals with the approach that we can stop the spread. This approach around the country has had no effect on containment. Future cases have always been found in these areas and elsewhere in those states. Eliminating as many animals as can be found in the positive testing areas makes the general public aware something is being done by the state agencies involved. The problem is this approach has been proven to have no influence on containment and controlling future cases.
>
> In many states that have domestic cervid, where CWD has been identified, there is almost always blame placed on domestic producers. Managing government agencies again believe adding more restraints, more regulation proves they are doing something. Many of these actions make no sense or have any science to back them up.
>
> The fact is producers have nothing to do with what has happened in the wild population in Idaho. Here in Idaho additional rules are being proposed for producers to again be the only ones that will suffer and be influenced by being taxed and regulated more than is already in place. Producers have been testing at a much higher rate than the rate of free ranging herds and we don't have CWD. If the Fish and Game had been testing at the same rate, they would have found CWD in the wild population long before now.
>
> We as producers have taken care of business. We have complied with the massive amounts of regulations, more than any other animal industry. We already are ahead of the curve. We have already been doing all the things that are necessary to be protected and more. With 100% testing of any death,

no matter the cause and 10% random testing of any healthy animal harvested is more than sufficient to catch and contain any CWD that might enter domestic herds. To require sampling of healthy animals to 100% adds a 90% increase cost to an already overly taxed system. Some think it's a small percentage of the value of an animal. The fact is that a large percentage of domestic cervid are used for meat production. At around $4/lb (hot hanging weight), an animal will bring between $600 to $900 per head. To add $50 plus cost of extracting samples to each animal is a cost that cannot be added and still hope to be profitable.

No other animal industry, even those known to have the same prion as CWD, have been required to do anything close to what we as domestic producers are required to do.

Don't punish us with more burden when we are already doing more than we should have to.
We have been asked by Department of Animal Industry to present evidence that proves we don't need additional testing in areas recognized to have CWD in free ranging herds. What more evidence do we need than a clean track record in an industry that has been operating in Idaho since the 1990s? Pretty good case history. Because we are just now finding these positive cases in wild population doesn't mean it hasn't existed. We believe it has and domestic producers have never contacted it here in Idaho.

Since CWD has been discovered across the country, there has been a massive amount of regulations placed on domestic cervid producers. Because of these regulations the numbers of cervid producers in Idaho have plummeted. This amazing opportunity for rural communities to use their private land is being killed by regulations.
We don't have many new cervid producers starting up. People look at the taxation and regulation's we have now and it's just too much government for most and just not worth it.

For those of us that have given our lives to this Industry, we are holding on but I don't know how we can just keep adding more restrictive rules without killing the industry.

The history of what we know about CWD has proven that doing more, isn't better. Let's not get caught up in the moment and put more burden on an already burdened industry.

We strongly oppose any additional regulations. We have more than we should have now. We urge Department of Animal Industry to please not add regulations that will do nothing but burden an already over regulated industry. Thanks for your consideration, Rulon Jones. Broadmouth Canyon Ranch

Sent from my iPhone

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BRAD LITTLE, GOVERNOR
CELIA GOULD, DIRECTOR

Domestic Cervidae Rulemaking Analysis

Docket No. 02-0419-2201

Background

The Idaho State Department of Agriculture (ISDA) received a Petition to Initiate Rulemaking from the Idaho Wildlife Federation (IWF) on March 8, 2022. The petition requested changes to testing requirements for domestic cervidae operations in proximity to detections of Chronic Wasting Disease (CWD) in wild cervids. IWF requested changes to IDAPA 02.04.19 Section 500.02 to require increased CWD testing for facilities *"within (25) miles from a confirmed case of CWD in wild cervids".*

The Rules Governing Domestic Cervidae were open for Zero Based Rulemaking in 2021 and were approved by the 2022 Legislature. The issue brought forward by IWF was not requested during 2021 rulemaking, but that rulemaking was conducted prior to the detection of CWD in wild cervids in Idaho.

The ISDA submitted the IWF's petition for rulemaking to the Department of Financial Management and the Governor's Office. The ISDA then initiated rulemaking in accordance with § Idaho Code 67-5230.

Rulemaking Authority

Domestic cervidae production is governed by Title 25, Chapter 37, Idaho Code. The authorizing statute requires CWD testing on all brain tissue samples from no less than 10 percent of all domestic cervidae 16 months of age or older that die or are harvested on domestic cervidae farms. § I.C. 25-3704A.

ISDA has authority to promulgate and enforce rules related to "registration of domestic cervidae farm or ranch premises, and for the prevention of the introduction or dissemination of diseases among domestic cervidae of this state, and to otherwise effectuate enforcement of the provisions of chapters 2, 3, 4, 6 and 37, title 25, Idaho Code, applicable to domestic cervidae." § I.C. 25-3704. The current rule language is consistent with the authorizing statute.

Negotiated Rulemaking

The ISDA facilitated public negotiated rulemaking meetings in May and June 2022. There was broad participation with the IWF, multiple cervidae producers from across the state, the Idaho Conservation League (ICL), the Theodore Roosevelt Conservation Partnership, and the North American Elk Breeders Association. The rulemaking process is meant to facilitate stakeholder input and development of consensus-based recommendations. Rulemaking participation is summarized in this analysis, and full rulemaking information is available on the ISDA website.

The IWF and the ICL commented in favor of the requested rule change. They are very concerned about the detection of CWD in wild Idaho cervids and see cervidae operations as a risk to wild cervid populations. They are concerned about the importation of cervids, especially those imported from other states or countries that are known to have CWD. ICL indicated that the proposed rule change presented a "relatively low cost on the whole," and "an ounce of prevention is worth a pound of cure" to address the spread of CWD.

Producers and elk industry representatives were against the proposed change. They expressed a concern about the level of testing that the Idaho Department of Fish and Game (IDFG) is performing on wild cervids. Some in the industry expressed the opinion that CWD is likely more prevalent in the wild than has been identified. Second, producers argued that it would be an unfair imposition for producers to test 100% of their animals because of the geographic proximity to CWD in wild populations. They argued that this would cause significant cost to be borne by the producers for a problem in wild populations. Third, the industry sees the 25-mile radius as arbitrary and not founded on a science-based standard or operational logic. They also stated that surveillance and testing for CWD by producers is above what is being done in wild populations.

IDFG participated in the rulemaking. Tricia Hebdon, IDFG Assistant Wildlife Chief, answered questions and offered technical information throughout the discussion. IDFG surveillance in wildlife populations has been ongoing since 1999, with IDFG collecting over 25,000 samples. In the last several years, IDFG started a rotational surveillance plan, with a focus on certain geographic areas. The current plan has focused on Idaho's eastern borders with Montana and Wyoming. IDFG believes that surveillance is working, and the disease has been identified to have a very low prevalence in Idaho, likely below two percent. In states where CWD prevalence increased, declines in wildlife populations were observed. This year, mandatory testing for all harvested wild cervids is in place for Game Management Units 14 and 15. IDFG is encouraging surrounding areas to be sampled voluntarily. IDFG is expecting over 5,000 samples from animals harvested in those two units.

Analysis

Through amendments to Idaho Code, the Legislature has provided direction on and requirements for CWD testing in domestic cervidae. The current CWD requirements in Idaho Code were enacted in 2014 with passage of H.B. 431. Prior to that, CWD testing was required on 100 percent of domestic cervidae 16 months or older that died on an Idaho operation. H.B. 431 decreased that to "no less than 10 percent." Hearings on the bill brought together a very similar stakeholder group to what ISDA had with this rulemaking. During the 2014 hearings, legislators discussed other CWD testing thresholds. In House Agricultural Affairs, a substitute motion was made to amend the bill's language from a 10 percent requirement to 70 percent. That motion failed on a voice vote, and the committee sent the bill to the House floor with a Do Pass recommendation. It passed the Legislature, was signed by the Governor, and went into effect on March 6, 2014. The "no less than 10 percent of harvested animals" language was adopted into the current Rules Governing Domestic Cervidae and remains the standard today.

CWD testing also was considered during 2022 Idaho Legislature. ISDA presented ZBR amendments to the Rules Governing Domestic Cervidae before the House and Senate Agriculture Affairs Committees. ISDA did not propose changes to the rule's threshold for CWD testing, but the agency finalized the rulemaking prior to the discovery of CWD in wild Idaho cervids. CWD was detected in Idaho prior to when the 2022 Legislature heard the ISDA's rule, and both committees approved the rule as presented. CWD detection and testing also was discussed outside of committee hearings through stakeholder discussions with lawmakers. No new legislation was introduced.

During this current rulemaking, all stakeholders shared concern about the detection of CWD in wild Idaho cervids and about the potential impact for wild and domestic populations alike. However, they have very different views about the risk posed by Idaho's domestic cervidae industry and about the burden that should be assigned to producers.

Cervidae producers voiced concerns about being singled out again as a primary threat for CWD when they feel they already do a significant amount of testing and observation for CWD. Wildlife advocates and conservation groups are concerned about domestic cervidae operations being a potential threat to wild cervid populations, especially if those operations import cervids from areas outside of Idaho known to have endemic CWD.

During the rulemaking discussion, producers expressed concerns that IWF did not seek out discussions with the cervidae industry before requesting a rule change. Producers indicated there may be some common ground, but they stated they were not consulted prior to this rulemaking. This remains an area to be explored outside of the rulemaking process.

While the meeting had good participation and strong discussion, stakeholders did not reach any consensus-based recommendations. Additionally, the agency did not receive new direction from the Legislature in 2022 through new legislation or rejection of the agency's rules.

Conclusion

ISDA has reviewed the requested change, stakeholder positions, and existing regulatory authority. With a careful balance of all factors, the ISDA is not moving forward with additional negotiated rulemaking, and the agency will not propose changes to the current Rules Governing Domestic Cervidae.

ISDA appreciates the diligence, professionalism, and courteousness shown by all stakeholders throughout the rulemaking process. We also understand that stakeholders have deep concerns about CWD and potential risks. ISDA remains committed to providing technical information if this issue is taken up by policymakers in the future.

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BRAD LITTLE, GOVERNOR

CELIA GOULD, DIRECTOR

"(8) The requirements of this section shall apply to the director's promulgation of new rules as well as the amendment, extension, or renewal of rules in effect on the effective date of this act."

1. Is this a ☐ new rule or ☑ amendment to current rule?

2. Is the proposed rule broader in scope or more stringent than federal law or regulations, or does it propose to regulate an activity not regulated by the federal government? ☑ Yes ☐ No
   a. If yes, which portions of the proposed rule?

   IDAPA 02.04.19 "Rules Governing Domestic Cervidae" contains the following sections that are either broader in scope or more stringent than federal law: 02.04.19.013-022, 02.04.19.030-305, 02.04.19.450-990.

3. Is the proposed rule pursuant to:
   a. Title 22, Chapter 49 (Beef Cattle Environmental Control Act)? ☐ Yes ☑ No
   b. Title 25, Chapter 38 (Ag Odor Management Act)? ☐ Yes ☑ No
   c. Title 37, Chapter 4 (Sanitary Inspection of Dairy Products) ☐ Yes ☑ No
   d. Title 37, Chapter 6 (Dairy Environmental Control Act) ☐ Yes ☑ No
   e. If yes to any of the above:
      i. List the peer-reviewed science and supporting studies (conducted in accordance with sound and objective scientific practices) utilized by the agency.

      ii. List the data that the agency utilized including site-specific, local, statewide, and regional data, including economic information.

iii. Explain how the rules are consistent with applicable legislative findings, policy, and intent; (for example, provide legislative bills or intent language).

iv. Has the agency made available for public review and comment, all scientific studies, (listed in subsection i. above) including underlying methodology, that have been relied upon by the director?

v. Have interested parties submitted economic feasibility data? ☐ Yes ☐ No
(Please attach data when submitting this document.)

4. Does the proposed rule propose a standard necessary to protect human health and the environment?
☐ Yes ☑ No If yes, Please complete subsections a-e. If no, please proceed to question 4.

a. Identify each population or receptor addressed by an estimate of public health effects or environmental effects.

b. Identify the expected risk or central estimate of risk for the specific population or receptor.

c. Identify each appropriate upper bound or lower bound estimate of risk.

d. Identify each significant uncertainty identified in the process of the assessment of public health effects or environmental effects and any studies that would assist in resolving the uncertainty.

e. Identify studies known to the agency that support, are directly relevant to, or fail to support any estimate of public health effects or environmental effects and the methodology used to reconcile inconsistencies in the data.

5. Does the notice for the proposed rule include information that the rule is boarder in scope or more stringent than federal law or regulations, or does it propose to regulate an activity not regulated by the federal government?

Yes ☑ No ☐

The proposed rule 02.04.19 is either broader in scope or more stringent than federal law. The program provides annual facility inspections, entry permits, and disease surveillance.

Information Compiled by: Dr. Scott Leibsle

Title: Administrator - Animal Industries

Date: 9.8.21

02.04.19 – RULES GOVERNING DOMESTIC CERVIDAE

000. LEGAL AUTHORITY.
This chapter is adopted under the legal authority of Sections 25-203, 25-305, 25-601, and 25-3704, Idaho Code. ( )

001. SCOPE.
These rules govern procedures for the detection, prevention, control and eradication of diseases among domestic cervidae, and facilities, record keeping, and reporting requirements of domestic cervidae ranches. ( )

002. – 003. (RESERVED)

004. INCORPORATION BY REFERENCE.
The following documents are incorporated by reference. ( )

01. Bovine Tuberculosis Eradication, Uniform Methods and Rules, Effective January 1, 2005. This document can be viewed online at https://www.aphis.usda.gov/animal_health/animal_diseases/tuberculosis/downloads/tb-umr.pdf. ( )

02. Code of Federal Regulations, Title 9, Part 161, January 1, 2021. This document can be viewed online at https://www.govinfo.gov/content/pkg/CFR-2016-title9-vol1/pdf/CFR-2016-title9-vol1-chapI-toc-id4.pdf. ( )

03. Code of Federal Regulations, Title 9, Part 55, January 1, 2021.This document can be viewed online at https://www.govinfo.gov/content/pkg/CFR-2016-title9-vol1/pdf/CFR-2016-title9-vol1-chapI-toc-id4.pdf. ( )

04. Code of Federal Regulations, Title 9, Subchapter A, Part 1 and 2, January 1, 2021.This document can be viewed online at https://www.govinfo.gov/content/pkg/CFR-2016-title9-vol1/pdf/CFR-2016-title9-vol1-chapI-toc-id4.pdf. ( )

005. -- 009. (RESERVED)

010. DEFINITIONS.

01. Approved Laboratory. NVSL, an AAVLD accredited laboratory that is qualified to perform CWD diagnostic procedures, or a laboratory designated by the Administrator to perform CWD diagnostic procedures. ( )

02. Approved Slaughter Establishment. A USDA inspected slaughter establishment at which ante-mortem and post-mortem inspection is conducted by USDA inspectors. ( )

03. Area Veterinarian in Charge. The USDA/APHIS/VS veterinary official who is assigned to supervise and perform official animal health activities in Idaho. ( )

04. Breed Associations and Registries. Organizations maintaining permanent records of ancestry or pedigrees of animals, individual animal identification records and records of ownership. ( )

05. Cervid Herd. One (1) or more domestic cervidae or groups of domestic cervidae maintained on common ground or under common ownership or supervision that may be geographically separated but can have interchange or movement. ( )

06. Cervidae. Deer, elk, moose, caribou, reindeer, and related species and hybrids including all

members of the cervidae family and hybrids. ( )

07. Chronic Wasting Disease. A transmissible spongiform encephalopathy of cervids that is a nonfebrile, transmissible, insidious, and degenerative disease affecting the central nervous system of cervidae. ( )

08. Commingling. Within the last five (5) years, the animals have had direct contact with each other, had less than thirty (30) feet of physical separation, or shared management equipment, pasture, or surface water sources, except for periods of less than forty-eight (48) hours at sales or auctions when a state or federal animal health official has determined such contact presents minimal risk of CWD transmission. ( )

09. Custom Exempt Slaughter Establishment. A slaughter establishment that is subject to facility inspection by USDA, but that does not have ante-mortem and post-mortem inspection of animals by USDA inspectors. ( )

10. CWD-Adjacent Herd. A herd of domestic cervidae occupying premises that border a premises occupied by a CWD positive herd, including herds separated by roads or streams. ( )

11. CWD-Exposed Animal. A cervid animal that is not exhibiting any signs of CWD, but has had contact within the last five (5) years with cervids from a CWD-positive herd or the animal is a member of a CWD-exposed herd. ( )

12. CWD-Exposed Herd. A herd of cervidae in which no animals are exhibiting signs of CWD, but: ( )

a. An epidemiological investigation indicates that contact with CWD positive animals or contact with animals from a CWD positive herd has occurred in the previous five (5) years; or ( )

b. A herd of cervidae occupying premises that were previously occupied by a CWD positive herd within the past five (5) years as determined by the designated epidemiologist; or ( )

c. Two (2) herds that are maintained on a single premises even if they are managed separately, have no commingling, and have separate herd records. ( )

13. CWD-Positive Cervid. A domestic cervid on which a diagnosis of CWD has been confirmed through positive test results on any official cervid CWD test by an approved laboratory. ( )

14. CWD-Positive Herd. A domestic cervidae herd in which any animal(s) has been diagnosed with CWD, based on positive laboratory results, from an approved laboratory. ( )

15. CWD-Suspect Cervid. A domestic cervid for which laboratory evidence or clinical signs suggests a diagnosis of CWD. ( )

16. CWD-Suspect Herd. A domestic cervidae herd in which any animal(s) has been determined to be a CWD-suspect. ( )

17. Death Certificate. A form, approved by the administrator, provided by the Division for the reporting of cervidae deaths and for reporting sample submission for CWD testing. ( )

18. Designated Epidemiologist. A state or federal veterinarian who has demonstrated the knowledge and ability to perform the functions required under these rules and who has been selected by the Administrator to fulfill the epidemiology duties relative to the state domestic cervidae disease control program. ( )

19. Disposal. Final disposition of dead cervidae. ( )

20. Domestic Cervidae. Fallow deer (*Dama dama*), elk (*Cervus elaphus*) or reindeer (*Rangifer*

tarandus) owned by a person. ( )

21. Domestic Cervidae Ranch. A premises where domestic cervidae are held or kept, including multiple premises under common ownership. ( )

22. Electronic Identification. A form of unique, permanent individual animal identification such as radio frequency identification tag, radio frequency identification implant, or other forms approved by the Administrator. ( )

23. Endemic Area. A geographical area designated by a state animal health official in the state of origin where animals located within that area are subject to an increased risk of acquiring a contagious disease. Most commonly in reference to Tuberculosis or Chronic Wasting Disease. ( )

24. Escape. Any domestic cervidae located outside the perimeter fence of a domestic cervidae ranch and not under the immediate control of the owner or operator of the domestic cervidae ranch. ( )

25. Federal Animal Health Official. An employee of USDA/APHIS/VS who is authorized to perform animal health activities. ( )

26. Harvest. Any healthy domestic cervid that is intentionally and lethally removed from a domestic cervidae facility, by an owner, designated employee or customer of the facility, strictly for the purposes of either shooting or meat production. Harvested includes cervids slaughtered at an approved or custom-exempt slaughter establishment. ( )

27. Herd of Origin. A cervid herd, on any domestic cervidae ranch or other premise, where the animals were born, or where they were kept for at least one (1) year prior to date of shipment. ( )

28. Herd Status. Classification of a cervidae herd with regard to CWD. ( )

29. Intrastate Movement Certificate. A form approved by the Administrator, and available from the Division, to document the movement of domestic cervidae between premises within Idaho. ( )

30. Individual CWD Herd Plan. A written herd management agreement and testing plan developed by the herd owner and approved by the Administrator to identify and eradicate CWD from a positive, source, suspect, exposed, or adjacent herd. ( )

31. Limited Contact. Incidental contact between animals of different herds in separate pens off of the herd's premises at fairs, shows, exhibitions and sales. ( )

32. National CWD Herd Certification Program. A federal-state-industry cooperative program administered by APHIS and implemented by participating states that establishes CWD surveillance and testing standards that owners must achieve before interstate transport of cervids will be permitted. ( )

33. Official CWD Test. A test approved by the Administrator and conducted at an approved laboratory to diagnose CWD. ( )

34. Official Identification. Identification, approved by the Administrator, that individually, uniquely, and permanently identifies each cervid. ( )

35. Operator. A person who has authority to manage or direct a domestic cervidae ranch. ( )

36. Premises. The ground, area, buildings, and equipment utilized to raise, propagate, control, or harvest domestic cervidae. ( )

37. Quarantine. An order issued on authority of the Administrator, by a state or federal animal health official or accredited veterinarian, prohibiting movement of cervids from any location without a written restricted

movement permit. ( )

38. Quarantine Facility. A confined area where selected domestic cervidae can be secured and isolated from all other cervidae and livestock. ( )

39. Ranch Management Plan. A written plan for a domestic cervidae ranch that sets forth best management practices that mitigates the introduction or dissemination of disease among domestic cervidae. ( )

40. Reidentification. The identification of a domestic cervid which had been officially identified, as provided by this chapter, but which has lost the official identification device, or the tattoo or official identification device has become illegible. ( )

41. Restrain. The immobilization of domestic cervidae in a chute, other device, or by other means for the purpose of efficiently, effectively, and safely inspecting, treating, vaccinating, or testing. ( )

42. Restricted Movement Permit. An official document that is issued by the Administrator, AVIC, or an accredited veterinarian for movement of animals from positive, suspect, or exposed herds. ( )

43. Source Herd. The herd or herds from where a producer acquired their existing livestock. ( )

44. State Animal Health Official. The Administrator, or Administrator's designee. ( )

45. Status Date. The date on which the Administrator approves in writing a herd status change with regard to CWD. ( )

46. Trace Back Herd. An exposed herd in which at least one (1) CWD positive animal resided within any of the previous sixty (60) months prior to diagnosis with CWD. ( )

47. Trace Forward Herd. A herd that has received exposed animals from a positive herd within sixty (60) months prior to the diagnosis of CWD in the positive herd or from the identified point of entry of CWD into the positive herd. ( )

48. Traceback. The process of identifying the movements and the herd of origin of CWD positive, or exposed animals, including herds that were sold for slaughter. ( )

49. Wild Cervidae. Any cervid animal not owned by a person. ( )

50. Wild Ungulate. Any four (4) legged, hoofed herbivore, including cervids and other ruminants, not owned by a person. ( )

51. Wild Ungulate Cooperative Herd Plan. A plan, developed cooperatively by the owner of the domestic cervidae ranch, the ISDA, and the Idaho Department of Fish and Game to determine the disposition of any wild ungulates that are found to be located on a domestic cervidae ranch. ( )

011. ABBREVIATIONS.

01. AAVLD. American Association of Veterinary Laboratory Diagnosticians. ( )

02. APHIS. Animal and Plant Health Inspection Service. ( )

03. AVIC. Area Veterinarian in Charge. ( )

04. AZA. Association of Zoos and Aquariums. ( )

05. CFR. Code of Federal Regulations. ( )

06. **CWD**. Chronic Wasting Disease. ( )

07. **HCP**. Herd Certification Program. ( )

08. **ISDA**. Idaho State Department of Agriculture. ( )

09. **NAEBA**. North American Elk Breeders Association. ( )

10. **NVSL**. National Veterinary Services Laboratory. ( )

11. **TB**. Tuberculosis. ( )

12. **UM&R**. Uniform Methods and Rules. ( )

13. **USDA**. United States Department of Agriculture. ( )

14. **VS**. Veterinary Services. ( )

012. APPLICABILITY.
These rules apply to all domestic cervidae located in, imported into, exported from, or transported through the state of Idaho. ( )

013. -- 019. (RESERVED)

020. LOCATION OF DOMESTIC CERVIDAE.
Any person who owns or has control of domestic cervidae in Idaho that are not located on a domestic cervidae ranch that is in compliance with the applicable provisions of this chapter is in violation of these rules. ( )

01. **Department Action**. In addition to any other administrative or civil action, the department may seize, require removal from the state, require removal to a domestic cervidae ranch that is in compliance with the provisions of this chapter, or require disposal of any domestic cervidae that are not located on a domestic cervidae ranch, an AZA accredited facility, or a USDA licensed facility which is in compliance with the provisions of this chapter. ( )

02. **Exceptions**. The Administrator may grant exceptions from the provisions of Section 020 on a case specific basis. ( )

03. **Natural Disasters**. Damage caused to domestic cervidae ranch facilities by natural disasters does not constitute a violation of this chapter, provided that the owner or operator begins any necessary repairs immediately upon discovering the damage, acts expeditiously, as determined by the Administrator, to complete any necessary repairs and reports the extent and cause of any damage to the Division within twenty-four (24) hours of the discovery of the damage. ( )

04. **Notification of Temporary Exhibition**. Producers must notify ISDA, in advance, of any event where a reindeer will be exhibited outside of an approved cervidae facility. ISDA must be provided with the date and location of the event as well as a description of the temporary facility and an escape plan protocol. ( )

021. OFFICIAL IDENTIFICATION.
All domestic cervidae must be individually, permanently, and uniquely identified, with two (2) types of official identification approved by the Administrator. ( )

01. **Reporting of Identification**. The unique individual identification number, type of identification, and the name, address, and telephone number of the owner of each animal identified must be reported to the Administrator, in writing, by the owner or operator. ( )

02. Identification Assigned. Official identification, once assigned to an individual animal, may not be changed or transferred to another animal. Animals that lose identification devices must be re-identified in accordance with Section 031. ( )

03. Progeny. All progeny of domestic cervidae must be officially identified by December thirty-first of the year of birth, upon sale or transfer of ownership, or upon leaving the domestic cervidae ranch, whichever is earlier. ( )

04. Visible Identification. At least one (1) of the official types of identification used must be visible from one hundred and fifty (150) feet. ( )

022. TYPES OF OFFICIAL IDENTIFICATION.

All domestic cervidae must be individually identified by two (2) of the following types of official identification, at least one (1) of the types of official identification must be a bangle or lamb tag that is visible from one hundred fifty (150) feet. ( )

01. Official USDA Ear Tag. ( )

02. Tattoo. Legible skin tattoo using an alphanumeric tattoo sequence that has been recorded with the Division of Animal Industries and applied to either the ear or escutcheon. ( )

03. Electronic Identification. A form of electronic identification, approved by the Administrator. ( )

04. Official NAEBA Eartag. ( )

05. Official ISDA Cervidae Program Ear Tag. A tamper resistant, unique number sequenced, individual identification tag approved by the Administrator. ( )

06. Official HASCO Brass Lamb Tag. A brass lamb tag engraved with farm name and individual animal identification number. ( )

07. Ranch Specific Unique Bangle or Lamb Tags. The Administrator may grant written approval for the use of bangle or lamb tags that are: ranch specific; tamper resistant; uniquely numbered; and correlated with another type of official identification on the annual inventory report. ( )

08. Other Identification. Other forms of unique individual identification approved by the Administrator. ( )

023. -- 029. (RESERVED)

030. OFFICIAL VISIBLE IDENTIFICATION.

01. Ear Tags. All domestic cervidae must be identified with a bangle or lamb tag that is visible from one hundred fifty (150) feet. ( )

02. Size. The large portion of the bangle or lamb tag must be at least two (2) square inches. ( )

03. Color. No visible identification may have a primary color of brown, black, pink, tan, or silver. ( )

04. Camouflage Patterns. No visible identification may utilize camouflage patterns. ( )

031. REIDENTIFICATION OF DOMESTIC CERVIDAE.

Permanent official identification in domestic cervidae that has been lost or is no longer legible may be replaced only for the purpose to reestablish their original identity. ( )

01. Records. All animals that have been re-identified must be reconciled to their original identification on the annual ISDA inventory form, due on Dec. 31st of each year. ( )

032. -- 039. (RESERVED)

040. INSPECTIONS.

To prevent the introduction and dissemination, or to control and eradicate diseases, state and federal animal health officials are authorized to inspect cervidae records, premises, facilities, and domestic cervidae to ensure compliance with the provisions of this chapter and other state or federal laws or rules applicable to domestic cervidae. State and federal animal health officials must comply with the operation's biosecurity protocol so long as the protocol does not inhibit reasonable access to: ( )

01. Entry. Enter and inspect, at reasonable times, the premises of domestic cervidae ranches and inspect domestic cervidae. ( )

02. Access to Records. Review or copy, at reasonable times, any records that must be kept in accordance with these rules. ( )

041. -- 059. (RESERVED)

060. WILD CERVIDAE.

Wild cervidae may not be confined, kept, or held on a domestic cervidae ranch. ( )

01. Duty of Ranch Owner. It is the duty of owners of all domestic cervidae ranches to take precautions, and to conduct periodic inspections, to ensure that wild cervidae are not located within the perimeter fence of any domestic cervidae ranch. ( )

02. Notification of Administrator. All owners or operators of domestic cervidae ranches must notify the Administrator within twenty-four (24) hours of gaining knowledge of the presence of wild cervidae inside the perimeter fence of the domestic cervidae ranch. ( )

03. Failure to Notify the Administrator. The failure of any owner or operator of a domestic cervidae ranch to notify the Administrator of the presence of wild cervidae within the perimeter fence of a domestic cervidae ranch is a violation of this chapter. ( )

04. Idaho Department of Fish and Game. Upon receiving notification that wild cervidae are on a domestic cervidae ranch, the Administrator will notify the Idaho Department of Fish and Game. ( )

061. -- 069. (RESERVED)

070. SUPERVISION OF DOMESTIC CERVIDAE PROGRAM.

A department veterinary medical officer will provide routine supervision of the domestic cervidae program. ( )

071. -- 089. (RESERVED)

090. FEES.

01. Annual Assessment Fee. A fee, not to exceed ten dollars ($10) per head per year on elk or three dollars ($3) per head per year on fallow deer and reindeer, is hereby assessed on all domestic cervidae in the state to cover the cost of administering the program covered in these rules. The fee includes all domestic cervidae present at the ranch as of December 31. This fee is due January first of each year. The annual assessment fee may be reduced if program revenue accumulates to a balance of at least one hundred thousand dollars ($100,000) in excess of the projected annual cost of operating the program, as determined by the Department on July 1 of each year. ( )

02. Import, Export, and Movement Fees. The fees imposed in Section 25-3708(2) through (4), Idaho Code, are due no later than December 31 of each year. ( )

091. -- 099. (RESERVED)

100. DOMESTIC CERVIDAE RANCHES.
In order to prevent the introduction or dissemination of diseases, and to control or eradicate diseases, all domestic cervidae ranches must comply with the disease control, facility, and record keeping requirements and all other provisions of this chapter. Each separate premises where domestic cervidae are kept or held must comply with all of the provisions of this chapter. ( )

101. DOMESTIC CERVIDAE RANCH FACILITY REQUIREMENTS.
Prior to populating the facility with domestic cervids, all domestic cervidae ranches are required to have facilities that include, but are not limited to, perimeter fence, restraining system, gathering system, water system, and if required, a quarantine facility. ( )

01. Maintenance. All facilities must be maintained, at all times that domestic cervidae are present, to prevent the escape of domestic cervidae or ingress of wild cervidae. ( )

02. Inspections. To ensure compliance with this chapter, state or federal animal health officials will inspect all premises where domestic cervidae are, or will be, possessed, controlled, harvested, propagated, held, or kept. ( )

102. PERIMETER FENCE REQUIREMENTS.
A perimeter fence, completely enclosing the domestic cervidae ranch to be constructed of high-tensile, non-slip woven wire or other fencing material approved by the Administrator. ( )

01. Elk and Fallow Deer. For elk and fallow deer, the fence must be a minimum of eight (8) feet in height for its entire length at all times. ( )

02. Reindeer. For reindeer, fences constructed and approved prior to 2021 must be at least six (6) feet in height for its entire length at all times. All reindeer fences constructed and approved in 2021 or later must be at least eight (8) feet in height for its entire length at all times. ( )

03. Wire. The top two (2) feet of each fence may be smooth, barbed or woven wire (at least twelve and one-half (12-1/2) gauge) with horizontal strands spaced not more than six (6) inches apart. ( )

a. Wire must be placed on the animal side of the fence to prevent pushing the wire away from the posts. ( )

b. Wire must be attached to all posts at the top, bottom, and not more than eighteen (18) inches apart between the top and bottom of the wire. ( )

04. Posts. Wooden posts used in the perimeter fence must be at least butt-end treated with a commercially available preservative and have a minimum of four (4) inch top for line posts and a minimum of five (5) inch top for corner posts. Metal pipe posts must be a minimum of two and one-eighth (2-1/8) inches outside diameter with a three-sixteenths (3/16) inch wall thickness for line posts and two and seven-eighths (2-7/8) inches outside diameter with a seven thirty-seconds (7/32) inch wall thickness for corner posts. Posts must be spaced no more than twenty-four (24) feet apart, with stays, supports or braces as needed, and be placed in the ground a minimum of three (3) feet. ( )

05. Gates. Each domestic cervidae ranch must have gates that prohibit the escape of domestic cervidae or the ingress of wild cervidae. ( )

06. Fence Maintenance. Fences must be maintained, at all times that domestic cervidae are present, to prevent domestic cervidae from escaping or native wild cervidae from entering the enclosure. ( )

07. Exceptions. The Administrator may grant exceptions to the specifications in Section 102 on a case specific basis. ( )

103. GATHERING AND RESTRAINING SYSTEM.

Each domestic cervidae ranch must have a system for humanely and effectively gathering and restraining domestic cervidae for the purpose of inspecting, identifying, treating, or testing of animals by state or federal animal health officials. ( )

01. Gathering System. Each domestic cervidae ranch must have a system that facilitates the gathering of domestic cervidae so as to be able to move the domestic cervidae through the restraining system, at any time of the year that domestic cervidae are present. ( )

02. Restraining System. A system approved by the Administrator, to immobilize domestic cervidae for the purpose of efficient, effective, and safe handling for inspecting, treating, vaccinating, or testing. ( )

03. Exceptions. The Administrator may grant exceptions to the provisions of this section on a case specific basis. ( )

104. QUARANTINE FACILITY.

If animals are to be imported onto the domestic cervidae ranch, a quarantine facility, approved by the Administrator, must be provided for holding animals until any disease retesting is accomplished or other requirements are met. ( )

105. -- 199. (RESERVED)

200. RECORDS AND REPORTING.

01. Reports. Owners of domestic cervidae ranches must submit complete and accurate reports to the Administrator. Failure to submit complete and accurate reports within the designated time frames is a violation of this chapter. ( )

02. Records. All owners of domestic cervidae ranches, during normal business hours, must present to state or federal animal health officials, for inspection, review, or copying, any cervidae records deemed necessary to ensure compliance with the provisions of this chapter. ( )

03. Notification. State animal health officials will attempt to notify the owners or operators of domestic cervidae ranches, and premises where records are kept prior to any inspections. ( )

04. Emergencies. In the event of an emergency, as determined by the Administrator, the notification requirements of Section 200 may be waived. ( )

201. ANNUAL INVENTORY REPORT.

01. Inventory Report. All owners of domestic cervidae ranches must submit annually, to the Administrator, a complete and accurate inventory and summary report form of all animals held no later than December 31st of each year containing the following minimum information: ( )

a. Name and address of the domestic cervidae ranch. ( )

b. Name and address of the owner of the domestic cervidae ranch. ( )

c. Date the inventory was completed. ( )

02. Individual Domestic Cervidae. For each individual domestic cervidae that was located on the domestic cervidae ranch during the year for which the report is being made, the following information must be

provided: ( )

a. All types of official and unofficial identification; ( )

b. Species; ( )

c. Sex; and ( )

d. Age or year born. ( )

202. INVENTORY VERIFICATION.

01. Visible Identification. Individual animal identification verification may be accomplished by visually noting the unique official visible identification number or visually noting an unofficial visible identification number if the number is correlated with two (2) forms of official identification on the inventory submitted by the cervidae producer. The Administrator may, on a case by case basis, grant written permission for ranch specific unique bangle tags to be used for official identification. ( )

02. Duty to Gather and Restrain. It is the duty of the owner of each domestic cervidae ranch to gather and restrain any domestic cervidae that state or federal animal health officials determine are not readily identifiable for inventory verification purposes. The Administrator determines the suitability of the restraint system. ( )

203. (RESERVED)

204. ESCAPE OF DOMESTIC CERVIDAE.

It is the duty of each owner or operator of a domestic cervidae ranch to take all reasonable actions to prevent the escape of domestic cervidae from a domestic cervidae ranch. ( )

01. Notification of Escape. When any domestic cervidae escape from a domestic cervidae ranch, the owner or operator of the domestic cervidae ranch must notify the Administrator by phone, facsimile, or other means approved by the administrator within twenty-four (24) hours of the discovery of the escape. ( )

02. Duty to Retrieve Escaped Cervidae. It is the duty of each owner or operator of a domestic cervidae ranch to retrieve or otherwise bring under control all domestic cervidae that escape from a domestic cervidae ranch. ( )

03. Fish and Game. The Administrator will notify the Idaho Department of Fish and Game of each escape. ( )

04. Capture. In the event that the owner or operator of a domestic cervidae ranch is unable to retrieve escaped domestic cervidae in a timely manner, as determined by the Administrator, the Administrator may effectuate the capture of the escaped domestic cervidae to ensure the health of Idaho's livestock and wild cervidae populations. ( )

05. Failure to Notify. Failure of any owner or operator of a domestic cervidae ranch to notify the Administrator within twenty-four (24) hours of the discovery of an escape of domestic cervidae is a violation of this chapter. ( )

06. Taking of Escaped Domestic Cervidae. A licensed hunter may legally take domestic cervidae that have escaped from a domestic cervidae ranch only under the following conditions: ( )

a. The domestic cervidae has escaped and has not been in the control of the owner or operator of the domestic cervidae ranch for more than seven (7) days; and ( )

b. The hunter is licensed and in compliance with all the provisions of the Idaho Department of Fish

and Game rules and code. ( )

205. NOTICE OF DEATH.
All domestic cervidae that die on a ranch or are sent to slaughter must be reported to the Department except for calves that died prior to being reported on an annual inventory. ( )

01. Submission of Death Certificates. A complete and accurate copy of all CWD sample submission forms/death certificates must be submitted to the division on a form approved by the Administrator no later than Dec. 31st in the calendar year the animal died. ( )

206 – 207. (RESERVED)

208. INTRASTATE MOVEMENT CERTIFICATE.
All owners of domestic cervidae ranches who move cervidae, from one premises to another, including movement from one (1) premises to another premises owned, operated, leased, or controlled by the owner, within the state of Idaho must submit, to the Administrator, a complete and accurate intrastate movement certificate signed by the owner, no later than Dec. 31st in the calendar year the movement occurred. The intrastate movement report must be submitted to the division on a form approved by the Administrator. ( )

209. RANCH MANAGEMENT PLAN.

01. Mandatory Ranch Management Plan. Domestic cervidae ranches are required to develop and implement an approved ranch management plan if the ranch is found in violation of Sections 060, 204 or 500 of these rules. The ranch management plan must be completed and implemented within six (6) months of the disposition of the violation. For the ranch management plan, the Administrator will conduct a risk assessment considering the factors in Subsection 209.03. Failure to comply with the mandatory ranch management plan is a violation of these rules. ( )

02. Risk Assessment for Ranch Management Plans. The Administrator will conduct a risk assessment for each ranch management plan. A ranch management plan will not include a double fencing requirement but may require that double gates be installed. The Administrator will consider the following factors when conducting a risk assessment at a domestic cervidae ranch: ( )

a. Risk of egress. The risk of egress may be evaluated based on, but not limited to, history of domestic cervidae escape during the previous five (5) years, recovery rate of escaped domestic cervidae, length of time domestic cervidae were outside of the perimeter fence, annual average precipitation, topography, altitude and tree density. ( )

b. Risk of ingress. The risk of ingress may be evaluated on, but not limited to, history of ingress during the previous five (5) years, annual average precipitation, topography, altitude, tree density and proximity to wildlife migration corridors. ( )

c. Compliance with CWD sample submission. The Administrator may, based on a risk assessment of the facility, adjust the number of tissue sample submissions required under this rule. The adjustment will be based on, but not limited to, the following: ( )

i. Whether the domestic cervidae on the ranch have commingled with any domestic cervids of unknown CWD status. ( )

ii. Whether the domestic cervidae ranch has been in compliance with all requirements of Title 25, Chapter 35, Idaho Code, and these rules. ( )

iii. Whether the domestic cervidae ranch has had documented cases of ingress of wild cervids or egress of domestic cervidae within the eighteen (18) months prior to the risk assessment. ( )

210. -- 249. (RESERVED)

250. INTRASTATE MOVEMENT OF DOMESTIC CERVIDAE.
All live domestic cervidae moving from one premises to another premises within the state of Idaho must be officially identified, except calves during the year of birth accompanying their dam, and accompanied by: ( )

01. Intrastate Movement Certificate. All intrastate movements of live domestic cervidae, including movement from one (1) premises to another premises owned, operated, leased, or controlled by the same person, must be reported to ISDA on the annual inventory form, due Dec. 31st in the calendar year the movement occurred. ( )

251. -- 300. (RESERVED)

301. DUTY TO RESTRAIN.
It is the duty of the owner of each domestic cervidae ranch to gather and restrain domestic cervidae for testing when directed to do so in writing by the Administrator. The Administrator determines the suitability of the restraint system. ( )

302. TESTING METHODS.
The Administrator determines appropriate testing procedures and methods. ( )

303. -- 499. (RESERVED)

500. SURVEILLANCE FOR CWD.

01. Routine Surveillance. Brain tissue from domestic elk and reindeer sixteen (16) months of age or older at the time of death must be submitted annually to official laboratories for CWD testing as provided for in these rules, under the following conditions: ( )

a. No less than ten percent (10%) of cervids harvested or slaughtered. ( )

b. No less than one hundred percent (100%) of cervids that die for any reason other than slaughter or harvest. ( )

c. Tissues samples submitted to an official laboratory that are untestable or are given an indeterminate test result do not count towards the tissue submission requirement. ( )

d. Fallow deer are exempt from CWD testing. ( )

02. Enhanced Surveillance. Brain tissue from one hundred percent (100%) of all domestic elk and reindeer sixteen (16) months of age or older that die for any reason on a facility will be required to be tested for CWD for a period of sixty (60) months under the following conditions: ( )

a. A facility is within twenty-five (25) miles from a confirmed case of CWD in wild cervids.

b. A facility has imported cervids from a location within twenty-five (25) miles from a confirmed case of CWD in wild cervids. ( )

~~b~~c. A facility has received cervids via intrastate movement from a facility under enhanced CWD surveillance requirements at the time of the transfer. ( )

~~c~~d. The duration of the enhanced CWD surveillance requirements are based upon the most recent date of movement that meets the criteria listed in this section. ( )

501. COLLECTION OF SAMPLES FOR CWD TESTING.
Obex samples must be collected immediately upon discovery of the death of a domestic cervid. ( )

01. Non-Testable or Samples That Do not Contain Appropriate Tissues. The Administrator may conduct an investigation to determine if a domestic cervidae ranch is complying with the provisions of Section 500 if the owner or operator of a domestic cervidae ranch submits samples for CWD testing which cannot be identified to the animal of origin. ( )

02. Failure to Meet Annual CWD Tissue Submission Requirement. An owner or operator of a domestic cervidae ranch who fails to submit samples for CWD testing or who fails to meet the annual tissue submission requirements of this chapter, or both, is in violation of these rules, except the Administrator may approve, in writing, a variance from sample submission requirements on a case specific basis. ( )

502. OFFICIAL CWD TESTS.

01. Official Tests. Official tests for CWD, approved by the Administrator, include: ( )

a. Enzyme Linked Immunosorbent Assay (ELISA); ( )

b. Immunohistochemistry; and ( )

c. Negative Stain Electron Microscopy. ( )

02. Other Scientifically Validated Test. The Administrator may approve other scientifically validated laboratory or diagnostic tests to confirm a diagnosis of CWD. ( )

503. CWD STATUS.
CWD status is validated pursuant to the Federal CWD Herd Certification program standards. ( )

504. INVESTIGATION OF CWD.
An epidemiological investigation will be conducted on all CWD positive, suspect, and exposed animals and herds, herds of origin, source herds, all adjacent herds, and all trace herds as determined by the Administrator. ( )

01. Quarantine. All positive, suspect, and exposed herds or animals, herds of origin, adjacent herds, and herds having contact with positive or exposed animals must be quarantined; and ( )

02. Identification. CWD suspect and exposed animals must be identified and remain on the premises where they are found until they have met the provisions for release of quarantine established in this chapter, are destroyed and disposed of as directed by the Administrator, or are moved at the Administrator's direction on a restricted movement permit. ( )

505. DURATION OF CWD QUARANTINE.
Quarantines imposed because of CWD in accordance with this chapter remain in effect until one (1) of the following criteria are met: ( )

01. CWD Positive Herds. The quarantine may be released after the herd is completely depopulated as provided in Subsection 505.07, or after five (5) years of compliance with an individual herd CWD plan and all provisions of these rules, during which there was no evidence of CWD. ( )

02. CWD Suspect Herds. The quarantine may be released after the herd is completely depopulated as provided in Subsection 505.07, or after a minimum of five (5) years of compliance with an individual CWD herd plan and all provisions of these rules and during which there was no evidence of CWD, or an epidemiologic investigation determines that there is no evidence CWD exists in the herd as determined by the Administrator. ( )

03. Source Herds and Herds of Origin. The quarantine may be released after a minimum of five (5) years of compliance with an individual CWD herd plan and all provisions of these rules and during which there was no evidence of CWD, or an epidemiologic investigation determines that there is no evidence CWD exists in the herd and that the herd is not the source of infection as determined by the Administrator. ( )

04. Exposed Herds. The quarantine may be released after the herd is completely depopulated as provided in Subsection 505.07, or after a minimum of five (5) years of compliance with an individual CWD herd plan and all provisions of these rules and during which there was no evidence of CWD, or an epidemiologic investigation determines that there is no evidence CWD exists in the herd as determined by the Administrator. ( )

05. Adjacent Herds. The quarantine may be released when directed by the Administrator based upon an epidemiological investigation and in consultation with the designated epidemiologist. ( )

06. Fencing Requirements. Any owner of a domestic cervidae ranch who chooses to remain under quarantine for five (5) years must construct a second perimeter fence that meets the requirements for perimeter fence, as provided in Section 102, such that no domestic cervidae on the domestic cervidae ranch can get within ten (10) feet of the original exterior perimeter fence or as approved by the Administrator. ( )

07. Complete Depopulation. The quarantine may be released after: ( )

a. Complete depopulation of all cervidae on the premises as directed by the Administrator; and ( )

b. The premises have been free of all livestock as specified in an individual CWD herd plan approved by the Administrator; and ( )

c. The soil and facilities have been cleaned, treated, decontaminated, or disinfected as directed by the Administrator. ( )

08. Disposal of Positive or Exposed Cervidae. All CWD positive or exposed domestic cervidae must be disposed of as directed by the Administrator. ( )

506. -- 999. (RESERVED)

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BRAD LITTLE, GOVERNOR
CELIA GOULD, DIRECTOR

****DIRECTOR'S MEMO****

To: Administrative Rules Coordinator

From: Celia Gould, Director

Subject: Director's Memo on Petition for Rulemaking on IDAPA 02.04.19

ISDA received a Petition to Initiate Rulemaking from the Idaho Wildlife Federation (IWF) on March 8, 2022. The petition requests changes to testing requirements for domestic cervidae operations in proximity to detections of Chronic Wasting Disease (CWD) in wild cervid populations.

The Rules Governing Domestic Cervidae were open for Zero Based Rulemaking in 2021 and were approved by the 2022 Legislature. The issue brought forward in the petition was not requested during 2021 rulemaking, but that was before the detection of CWD in wild cervids last winter.

If approved, we would anticipate conducting negotiated rulemaking over two meetings in May. Stakeholders involved likely would include the Idaho Wildlife Federation as the petitioner, the domestic cervidae industry, the Idaho Department of Fish and Game, and others.

IDAHO STATE DEPARTMENT OF AGRICULTURE ("ISDA")
PETITION TO INITIATE RULEMAKING

All rulemaking petitions must substantially comply with IDAPA 04.11.01.820, which addresses petitions to initiate rulemaking as described by Section 67-5230, Idaho Code. The requirements have been laid out below. ISDA will consider all petitions and act to either initiate or deny rulemaking in accordance with I.C. § 67-5230(1) and IDAPA 04.11.01.821.

Please note that ISDA may only conduct rulemaking within the authority provided it by statute in order to govern the department's jurisdiction. *See* I.C. § 22-101(3). Prior to petitioning ISDA, please verify and understand the authority of ISDA as it relates to the petition's desired outcome. If a petition for rule change is outside of ISDA's legal authority, it will be denied.

Name of petitioner(s): Brian Brooks, Executive Director, Idaho Wildlife Federation

Address of petitioner: 1020 W Main Street, Suite 450, Boise, ID 83702

Phone number of petitioner: (208)342-7055

Email address of petitioner: bbrooks@idahowildlife.org

Petitioner's interest in matter:
Please see attached document.

Describe the nature of the rule or amendment to the rule and the petitioner's suggested rule or amendment: Please see attached document.

Name of the statute, order, rule or other controlling law:
Please see attached document.

Factual allegations upon which the petitioner relies to support the proposed rulemaking:
Please see attached document.

Citations of cases and/or statutory provisions that apply (optional):

Idaho State Department of Agriculture ("ISDA") Petition to Initiate Rulemaking

Name of petitioner(s): Brian Brooks, Executive Director, Idaho Wildlife Federation
Address of petitioner: 1020 W Main Street, Suite 450, Boise, ID 83702
Phone number of petitioner: (208)342-7055
Email address of petitioner: bbrooks@idahowildlife.org

Petitioner's interest in matter:
As Idaho's oldest and largest sporting organization, IWF has a long history in advocating for the health and viability of our state's wild deer and elk populations. Chronic Wasting Disease poses an immediate and long-term threat to both domestic and wild cervid populations. IWF is committed to minimizing these threats to the greatest extent practicable.

Describe the nature of the rule or amendment to the rule and the petitioner's suggested rule or amendment:
IWF petitions ISDA to increase surveillance for Chronic Wasting Disease by amending IDAPA 02.04.19, Section 500.02 to reflect the following language:

"Brain tissue from one hundred percent (100%) of all domestic elk and reindeer sixteen (16) months of age or older that die for any reason on a facility will be required to be tested for CWD for a period of sixty (60) months under the following conditions:
a. A facility is within twenty-five (25) miles from a confirmed case of CWD in wild cervids.
~~a~~b. A facility has imported cervids from a location within twenty-five (25) miles from a confirmed case of CWD in wild cervids.
~~b~~c. A facility has received cervids via intrastate movement from a facility under enhanced CWD surveillance requirements at the time of the transfer.
~~c~~d. The duration of the enhanced CWD surveillance requirements are based upon the most recent date of movement that meets the criteria listed in this section."

Name of statute, order, rule or other controlling law:
IDAPA 02.04.19. Rules Governing Domestic Cervidae
Section 500. Surveillance for CWD. Subsection 02. Enhanced Surveillance.

Factual allegations upon which the petitioner relies to support the proposed rulemaking:
Chronic Wasting Disease was detected in wild cervids for the first time in Idaho in Fall 2021. ISDA finalized the negotiated rulemaking for Rules Governing Domestic Cervidae before this detection in the wild was confirmed. Current rules only require enhanced surveillance and testing relating to interstate transport and therefore CWD from outside of Idaho's borders. Current rules do not consider the threat of CWD transmission from wild animals already in Idaho into domestic facilities. Artificial congregation and movement of domestic cervids as well as interaction between wild and domestic cervids will continue to facilitate the spread of CWD. It is necessary for ISDA to increase surveillance efforts for CWD in domestic cervidae now that it is present within Idaho's borders.

Citations of cases and/or statutory provisions that apply (optional):