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RT-QuIC detection of CWD prion seeding activity in white-tailed deer muscle tissues

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Chronic wasting disease (CWD) is a prion disease circulating in wild and farmed cervid populations throughout North America (United States and Canada), Europe (Finland, Norway, Sweden), and South Korea. CWD is a long-term threat to all cervid populations and to cervid hunting heritage, with the potential to cause substantial economic losses across multiple sectors. In North America, hunting and farming industries focused on the processing and consumption of white-tailed deer (WTD) venison are particularly vulnerable to CWD prion contamination, as millions of WTD are consumed annually. Real-time quaking-induced conversion (RT-QuIC) is a highly sensitive assay amplifying misfolded CWD prions in vitro and has facilitated CWD prion detection in a variety of tissues and excreta. To date, no study has comprehensively examined CWD prion content across bulk skeletal muscle tissues harvested from individual CWD infected WTD. Here, we use RT-QuIC to characterize prion-seeding activity in a variety of skeletal muscles from both wild and farmed CWD-positive WTD. We successfully detected CWD prions in muscles commonly used for consumption (e.g., backstrap, tenderloin, etc.) as well as within tongue and neck samples of WTD. Our results suggest that CWD prions are distributed across the skeletal muscles of infected WTD. We posit that RT-QuIC will be a useful tool for monitoring CWD prions in venison and that the method (with additional protocol optimization and high-throughput functionality) could be used to reduce and/or prevent CWD prions from entering animal and human food chains.

Chronic wasting disease (CWD) is an infectious and fatal prion disease transmitted among cervids, including white-tailed deer (WTD; *Odocoileus virginianus*), mule deer, elk, red deer, caribou, reindeer, and moose. The disease is a direct threat to a number of cervid-related multibillion-dollar economic sectors, including both agricultural and hunting industries, and it is now prevalent in the USA and Canada with additional cases in Korea, and Scandinavian regions[1]. As with other transmissible spongiform encephalopathies[2,3], CWD prion seeds (PrP^{CWD}) consist of misfolded cellular prion protein (PrP^{C}) which form β-sheet-rich amyloid fibrils through inducing conformational change and polymerization of native PrP^{C}. The central nervous system (CNS) typically contains the highest load of prions in a terminally diseased animal in comparison to peripheral tissues and body excreta due to the abundance of PrP^{C} in nervous tissues[4].

Recent studies have shown that there are compelling reasons to suggest that CWD poses a non-zero risk to a variety of mammals, including humans[1,5]. Challenge experiments using CWD prions have shown that CWD can cause neurodegenerative disease in numerous species, including ferrets, mink, domestic cats, sheep, goats, cows, pigs, and squirrel monkeys[6]. In vitro experiments showed that CWD prions can convert human prion proteins into a misfolded and potentially disease-causing form[5]. For these reasons, as of 2020, both the Food and Drug Administration (FDA) and Food Safety and Inspection Service, United States Department of Agriculture (FSIS, USDA) consider venison from CWD-positive animals as adulterated and unsuitable for consumption[7,8]. While there is no evidence of CWD transmission to humans[1], the National Institutes of Health and Centers for Disease Control and Prevention suggests that people should not consume known CWD-infected venison.

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Currently, CWD diagnosis relies on the identification of Proteinase K (PK)-resistant PrP^{CWD} by enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC)[9]. These standardized methods for detecting CWD are designed to have consistent protocols with quantified estimates of test accuracy that are scalable to meet the needs of agencies conducting surveillance and monitoring to manage the disease. However, there are limitations to the existing antibody-based diagnostic approaches, namely relatively poor sensitivity as well as the inability to screen biofluids and environmental samples. In the past two decades, the detection of prion seeding activity has been greatly enhanced by highly sensitive methods involving amplification of protein misfolding in vitro, such as protein misfolding cyclic amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC)[9,10]. PMCA uses rodent brain homogenates as the substrate to amplify misfolded prions and Western Blotting as the output[9,11,12]. RT-QuIC utilizes recombinant PrP^{C}, commonly from rodent sources, as the substrate for prion amyloid formation, the real-time reporting of which is enabled by thioflavin T (ThT) binding and detection[13–15]. Although RT-QuIC demonstrates unparalleled detection sensitivity and specificity for brain and lymphoid tissues, it has lower sensitivity for other sample types; this has in-part been ascribed to lower prion density and RT-QuIC reaction inhibitors[16]. Importantly, the inhibitory effect of certain tissues—likely due to their biochemical compositions[16,17], such as blood[18–20] and saliva, seemed to be specific for RT-QuIC but not PMCA[16]. Dilutions (diluting out inhibitory effects) and phosphotungstic acid (PTA) precipitation are commonly used to increase RT-QuIC sensitivity by enriching for prions and overcoming effects of RT-QuIC inhibitors[15–17,20], although other methods exist[18,21].

CWD prions have previously been identified in a variety of tissue types and excreta using RT-QuIC, such as the CNS, third eyelids, and feces[22–25]. Prior studies focused on the detection of CWD prions in skeletal muscle using immunodetection methods have produced mixed results[26–29]. PMCA was used to amplify CWD prions in hindlimb muscles from two WTD[30]. Skeletal muscle tissues from CWD-infected deer contain infectious prions as determined in transgenic mice bioassay[29]. Despite clear advantages of RT-QuIC as a screening method[9,31,32], no comprehensive reports are available for detecting CWD prions using RT-QuIC in skeletal muscle.

Cervid skeletal muscles are consumed by a growing population of hunters and restaurant clientele and have become a common ingredient in pet food (e.g., commercial cat and dog food). At the time of this publication, there are no guidelines regarding venison-based detection of CWD and associated food-product surveillance. This observation, combined with the limitations of existing CWD diagnostic tools (e.g., ELISA and IHC), has resulted in a situation whereby venison processing can occur without the knowledge of an animal's CWD status, and it is estimated that at least 15,000 CWD positive cervids are consumed in the USA annually[1]. Underscoring this statistic was a well-documented 2005 exposure event in which over 200 participants at a Sportsmen's feast consumed CWD-positive venison[33]. Current estimates indicate a 20–50% CWD prevalence rate in harvested WTD from focal areas of southern Wisconsin, however, only 1 out of 3 are tested for the disease[33]. Collectively, these observations highlight the need for post-harvest production-level monitoring of cervid products used for human and animal consumption.

Here, we examine the utility of RT-QuIC for the detection of CWD prions within a broad set of WTD skeletal muscle tissues, including those frequently used for both human and animal consumption. We report the RT-QuIC results for muscles sampled from the neck (*brachiocephalicus/sternocephalicus*) of wild WTD with known CWD status. Further, we investigated whether CWD prion deposition is limited to certain groups of muscles or if it is more generalized by using multiple WTD skeletal muscle groups across the body, including muscles from the tongue, forelimb (*suprascapularis*), backstrap (*longissimus dorsi*), tenderloin (*psoas major*), and hindlimb (*semimembranosus/semitendinosus*) from both wild and farmed CWD positive animals independently determined by ELISA and/or IHC.

Results

RT-QuIC detection of CWD prions in unilateral skeletal muscles from the neck of wild WTD. We first developed a PrP^{CWD} enrichment protocol for muscles—based on previous work[20,25]—herein referred to as the freeze–thaw method as it consists of several rapid freeze–thaw cycles prior to PTA precipitation. To test the performance of the freeze–thaw method, we processed unilateral muscles collected from the neck (*brachiocephalicus/sternocephalicus*) of 10 CWD-positive and 10 CWD-negative wild WTD (Table 1) and analyzed the resultant homogenates using RT-QuIC. We found that we could detect significant prion seeding activity in 8 out of 10 (80%) samples from 10 different CWD-positive animals (Table 1; Fig. 1a) with relatively consistent fluorescent readings (Fig. 1b). In contrast to animals with official CWD-positive test results (i.e., ELISA and IHC), none of the muscle samples from CWD-negative animals showed statistically significant prion seeding activity in RT-QuIC (Fig. 1b), despite one of eight wells crossing the threshold from a single animal (Fig. 1d).

We then compared the rate of amyloid formation (RAF) among muscles, blood, and lymphoid tissues, all of which were processed using mechanical extraction methods; with methods and results of blood and lymphoid tissues reported by Schwabenlander et al.[32]. We note that although muscles appeared to have a lower RAF, it is possible for an animal to have a statistically positive RT-QuIC result for muscles and lymphoid tissues but not blood (e.g., animal 166; Fig. 2a; see Schwabenlander et al. In press). 1:10 dilution of the enriched homogenates (after NaPTA precipitation) was chosen because of its consistency in producing results in different animals (Fig. 2b). The optimal dilutions (a log10-based dilution that would produce the highest RAF for a given sample) of each animal may differ, with dilution factors ranging from 0 to 3 (Fig. 2b). Because the initial concentration of prion seeds added into RT-QuIC reaction is known to affect detectability and RAF[15], it is then expected that the method presented here—using suboptimal dilutions for some samples—would underestimate the RAF for consistent detection purposes. Indeed, by comparing the RAF between 1:10 dilution of the enriched homogenates and lymphoid tissues, which exhibit RAF 10 times lower than brain homogenates[17], we found that indirectly calculated brain/muscle ratio was 100–1000 times lower than previously reported in muscles of mice inoculated

MNPRO ID	ELISA/IHC CWD test result	RT-QuIC result	RT-QuIC wells positive
166	+	***	7/8
250	+	**	6/8
333	+	**	6/8
353	+	*	5/8
360	+	****	8/8
363	+	*	5/8
376	+	***	7/8
384	+	***	7/8
508	+	NS	2/8
735	+	NS	1/8
443	–	–	0/8
239	–	–	0/8
515	–	–	0/8
536	–	–	0/8
693	–	NS	1/8
708	–	–	0/8
723	–	–	0/8
727	–	–	0/8
734	–	–	0/8
762	–	–	0/8

Table 1. RT-QuIC results of WTD neck muscles. All animals were collected through the Minnesota Department of Natural Resources 2019 agency culling operations. All animals' medial retropharyngeal lymph nodes were tested for CWD through official regulatory means by ELISA, with IHC confirmation on ELISA positives. Mann–Whitney U test: NS, rate of amyloid formation is not 0 but not statistically significant from the corresponding negative controls; –, rate of amyloid formation is 0 in the given time period; ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. The freeze–thaw method was used for sample processing and RT-QuIC was performed at 45 °C.

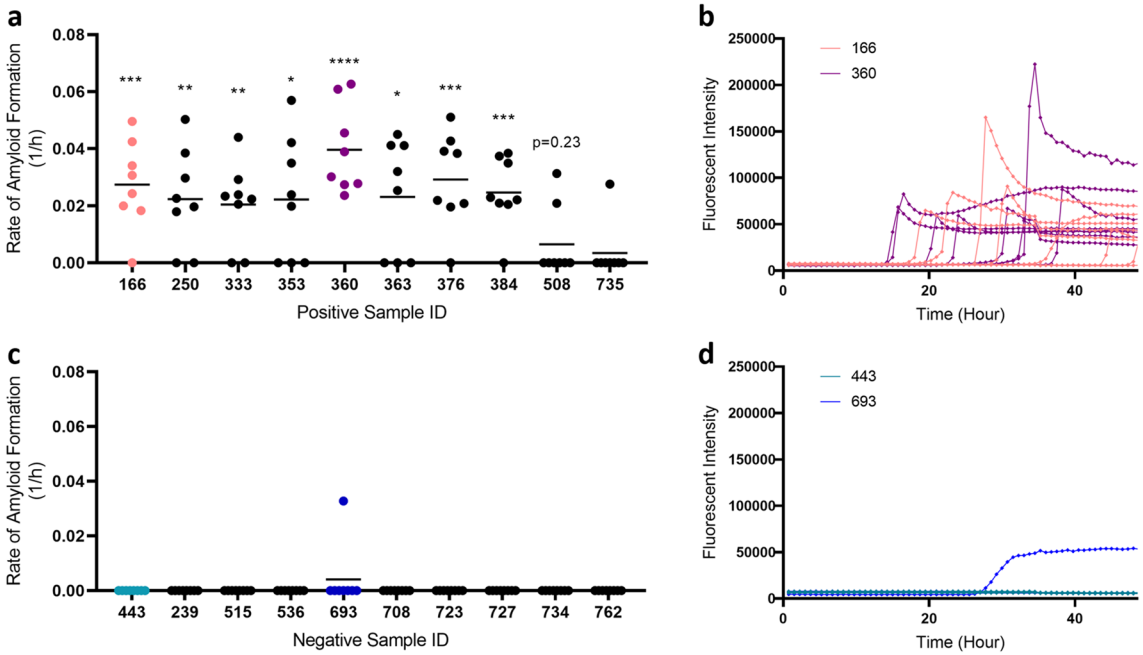

Figure 1. Detection of prion seeding activity in unilateral neck muscles from white-tailed deer. (**a**) Rate of amyloid formation (1/h) was plotted using data collected from CWD-positive animals. Statistical significance was obtained through comparing with rate of amyloid formation with the respective negative controls on the same plate (****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). (**b**) Examples of real-time fluorescence readings from positive animals (sample IDs 166 and 360). (**c**) Rate of amyloid formation (1/h) from CWD-negative animals. (**d**) Examples of real-time fluorescence readings from negative animals (443 and 693); plotted as described in (**b**) and showing one of eight wells for sample 693 having amyloid seeding activity (not significant).

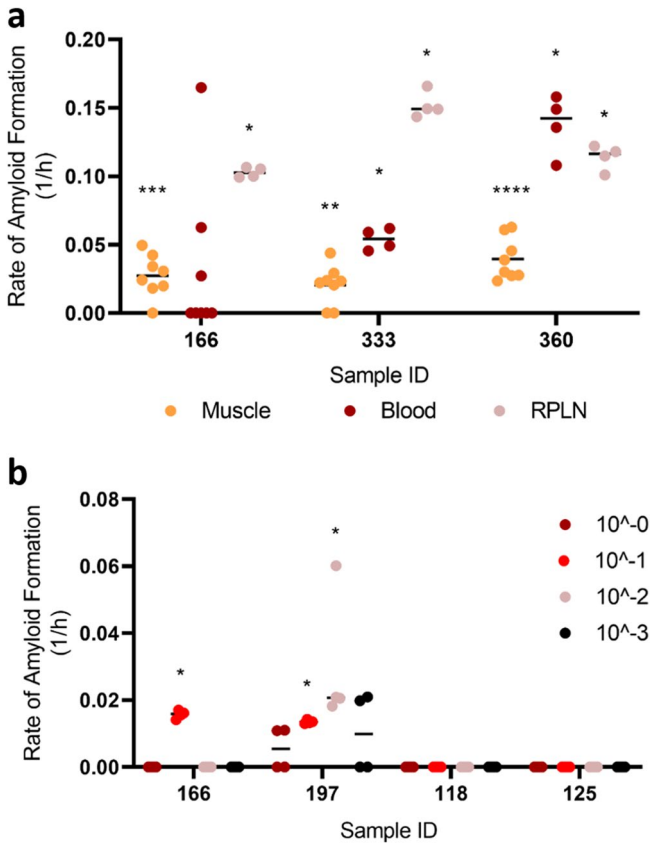

Figure 2. Comparison of prion-seeding activity in RT-QuIC. (**a**) Rate of amyloid formation was compared among neck muscle, blood, and lymphoid tissues of three CWD-positive animals. (**b**) Rate of amyloid formation compared between each sample and the negative control on the same plate. Statistical result for each sample compared with its respective negative control was indicated (****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). RPLN, medial retropharyngeal lymph nodes.

with different strains of prions (Supplementary Fig. 1)[34]. Because the tenfold dilution for each sample was likely not optimal, the difference could be attributed to a combination of the presence of RT-QuIC inhibitors in muscle tissues and incomplete extraction (Bosque et al. pulverized muscles under liquid nitrogen[34]) in addition to experimental design and species differences. It is also possible that particular CWD prion seeds within our samples were partially degraded during autolysis (discussed further below).

CWD prions found in muscles from the tongue, neck, mid-trunk, forelimb, and hindlimb of WTD. To investigate if the CWD prions are found in WTD skeletal muscles and whether the freeze–thaw method described above can be used to detect prions deposition in other skeletal muscles other than those from the neck, we used a set of muscle tissues from another 10 WTD, including the tongue, forelimb (*suprascapularis*), backstrap (*longissimus dorsi*), tenderloin (*psoas major*), and hindlimb (*semimembranosus/semitendinosus*). In the blinded run, we were able to detect at least one significantly RT-QuIC positive sample in all the muscle groups tested (Table 2; Fig. 3). We observed poor sensitivity of the freeze–thaw method with these particular samples (Table 2; when compared to fresh samples), a result that is likely due to the deteriorated condition of the muscle tissues upon receipt. Nevertheless, we recovered statistically significant RT-QuIC results for a variety of muscle groups and we therefore conclude that PrP^{CWD} occurs broadly throughout the skeletal muscles of infected WTD (Fig. 3b) and are not limited to specific muscle groups, as previously reported in mouse models[34].

Notably, the samples used for this experiment were undergoing various degrees of autolysis. Hypothesizing that this may influence RT-QuIC's ability to detect prion-seeding activity by changing the optimal dilutions of the processed homogenate, we again looked at prion-seeding activities using serial dilutions of a selected number of samples. As expected, the dilution with adequate positive wells for samples no longer consistently converged at 10^{-1} (Fig. 4a), suggesting that the freeze–thaw method is not suitable for muscle tissue samples of sub-optimal quality. To investigate whether other tissue processing methods would improve the detection of CWD prion-seeding activity in RT-QuIC, given the sub-optimal tissue preservation described above, we examined a subset of samples using enzymatic digestions (collagenase A and trypsin) instead of the freeze–thaw method. We hypothesized that collagenase A and/or trypsin would sufficiently digest potential inhibitors and/or further "release" CWD prions to a degree where extensive dilution of the processed homogenates was unnecessary. Surprisingly, collagenase A digestion still required a tenfold dilution similar to the freeze–thaw method (Supplementary

ID	Region	MNPRO ID	ELISA and/or IHC CWD test result	RT-QuIC result	RT-QuIC wells positive
1	F	287	+	–	0/8
2	H	287	+	NS	3/8
3	Tg	287	+	***	8/8
4	B	287	+	–	0/8
5	F	288	+	NS	1/8
6	H	288	+	NS	1/8
7	B	288	+	NS	1/8
8	F	289	+	NS	1/8
9	H	289	+	–	0/8
10	B	289	+	–	0/8
11	F	290	+	–	0/8
12	H	290	+	NS	2/8
13	Tg	290	+	–	0/8
14	Td	290	+	–	0/8
15	H	295	+	NS	1/8
16	F	295	+	–	0/8
17	B	295	+	NS	1/8
18	H	296	+	–	0/8
19	F	296	+	–	0/8
20	B	296	+	–	0/8
21	H	297	+	NS	1/8
22	F	297	+	NS	1/8
23	B	297	+	–	0/8
24	H	298	+	NS	1/8
25	F	298	+	–	0/8
26	B	298	+	–	0/8
27	H	307	+	***	8/8
28	F	307	+	****	8/8
29	B	307	+	*	4/8
30	Td	307	+	NS	1/8
31	H	311	+	–	0/8
32	F	311	+	–	0/8
33	B	311	+	–	0/8
34	Td	311	+	*	5/8

Table 2. RT-QuIC results of various WTD muscle groups. All animals' medial retropharyngeal lymph nodes were tested for CWD through official regulatory means by ELISA, with IHC confirmation on ELISA positives, except MNPRO ID 307, which was tested by IHC only on obex and medial retropharyngeal lymph nodes. Mann–Whitney U test: NS, rate of amyloid formation is not 0 but not statistically significant from the corresponding negative controls; –, rate of amyloid formation is 0 in the given time period; ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. RT-QuIC analyses of the forelimb (F), hindlimb (H), backstrap (B), tenderloin (Td), and tongue (Tg) muscles were performed with the researcher blinded to official CWD testing results (see methods). The freeze–thaw method was used for sample processing and RT-QuIC was performed at 45 °C.

Fig. 2) although it appeared to be more sensitive [i.e., identified more muscle samples as RT-QuIC positive from CWD positive animals (Fig. 4b)]; however, this was not observed when we re-tested a subset of neck muscle samples. In addition, we confirmed that the collagenase method did not appear to produce false-positive RT-QuIC signals (Supplementary Fig. 3). Alternatively, trypsin digestion produced an extremely high RAF without requiring the tenfold dilution even though its sensitivity did not improve upon the freeze–thaw method in the given sample set (Fig. 4c).

We note that all methods used in this study did result in positive prion-seeding activities using RT-QuIC on muscle tissue (Fig. 4b). The results reported here indicate that the freeze–thaw method may not be enough to facilitate RT-QuIC detection of CWD prions in aged or decomposing muscles but has utility for freshly collected samples.

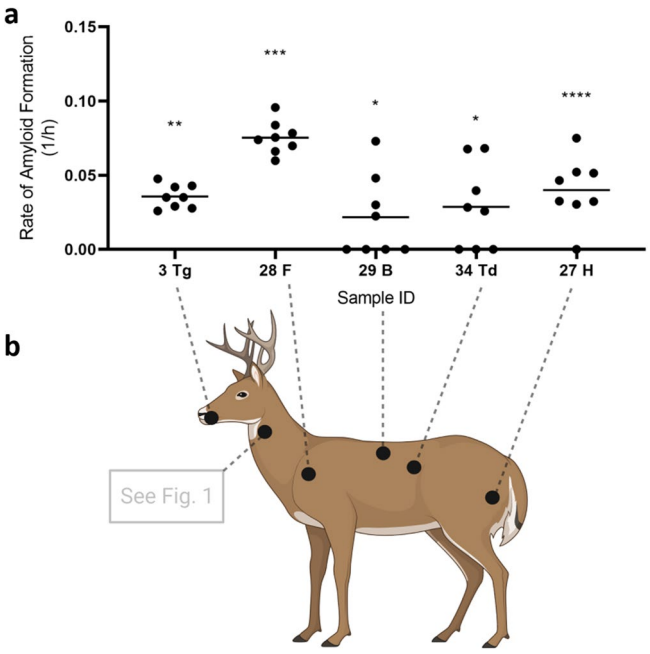

Figure 3. Presence of CWD prions in the muscle tissues of tongue, neck, hindlimb, forelimb, backstrap, and tenderloin. (**a**) Examples of the rate of amyloid formation (RAF) from RT-QuIC positive samples were plotted. Statistical results as compared to the respective negative controls were indicated (****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). *Tg* Tongue, *F* forelimb, *B* backstrap, *Td* tenderloin, *H* hindlimb. Numbers on X-axis are animal IDs listed in Table 2. (**b**) Prion-seeding activity was detected by RT-QuIC in muscles from the tongue, forelimb, backstrap, tenderloin, and hindlimb. Deer image was created using BioRender.com.

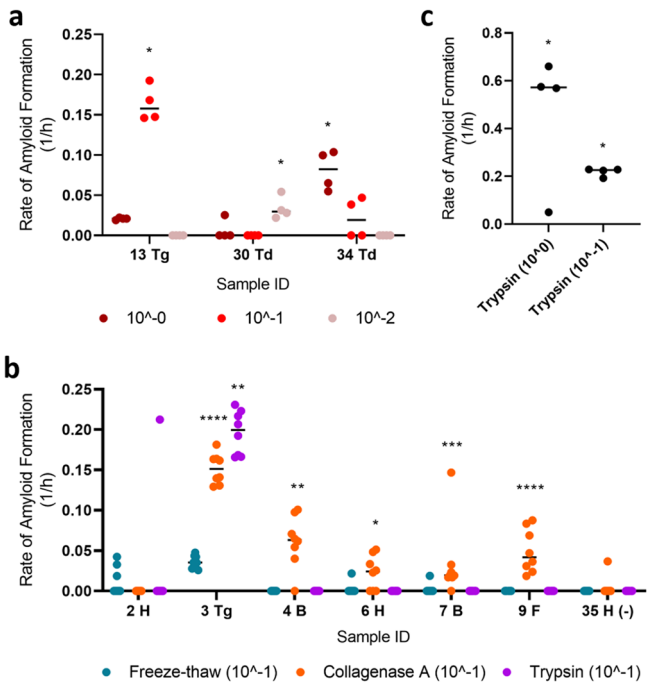

Figure 4. Comparison of different methods used to extract CWD prions from skeletal muscles of WTD. (**a**) Rate of amyloid formation (RAF) of samples diluted to different concentrations was visualized. (**b**) RAF from RT-QuIC was plotted for a subset of samples treated by different extraction methods, including freeze–thaw, collagenase A, and trypsin. The final suspension was diluted to 10^{-1}. (**c**) RAF of trypsin-digested sample number 3 (tongue; undiluted and diluted to 10^{-1}) was diagramed. Statistical result for each sample when compared with its respective negative control was indicated (****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). *B* backstrap, *F* forelimb, *H* hindlimb, *Td* tenderloin, *Tg* tongue.

Discussion

CWD is an emerging infectious prion disease currently affecting cervid populations across three continents and negatively influencing all cervid-related industries within impacted regions. Infected animals can remain asymptomatic for months while shedding CWD prions through excreta[22,25], thus making the identification of early-stage CWD-infected animals based on external diseased phenotypes impossible. Antibody-based ELISA and IHC tests are the current diagnostic standards for CWD. Despite their reliability, such immunodetection methods have limited sensitivity and application across various tissues and body excreta in comparison to in vitro amplification methods for prion detection, such as PMCA and RT-QuIC[9]. Of the available in vitro amplification methods, RT-QuIC is well-suited as a CWD screening tool because it can be easily scaled up as required by industrial applications. Given the continued spread of CWD, and uncertainty surrounding potential health risks to both animals and humans due to the consumption of CWD-positive venison[1], it is clear that a highly sensitive and reliable diagnostic method to detect CWD prions in skeletal muscles of cervids is needed.

In this study, we tested methods aimed at extracting and enriching PrP^{CWD} from WTD skeletal muscles for prion detection by RT-QuIC. We first found that CWD prions were present in bulk sampled neck muscles (*brachiocephalicus*/*sternocephalicus*) of CWD positive animals (Fig. 1a)[32]. This result prompted us to investigate the general distribution of prions in skeletal muscles from the tongue, forelimb, mid-truck, and hindlimb of CWD positive WTD tissue-sets available in our biorepository. We found that in addition to the neck, PrP^{CWD} is also present in a variety of skeletal muscle tissues (described above; Table 2; Fig. 3). Our results, based on a sampling of various muscle groups, suggest that CWD prions are distributed across CWD infected WTD skeletal muscles. Additional research is needed to determine the full extent to which CWD prions occur within particular muscle tissue types of infected animals, including intra- and inter-individual variation of CWD prion accumulation in WTD muscles.

It remains to be determined whether CWD prions are detectable in skeletal muscles that were not sampled herein or in similar studies using amplification-based methods, such as PMCA and RT-QuIC. Although we were unable to detect PrP^{CWD} across all muscle types within a given CWD positive animal, this result is expected because the successful detection of PrP^{CWD} within an infected individual, and particular tissue type, can be impacted by multiple factors. With respect to the neck samples screened here, we only had access to unilaterally sampled muscles harvested from individual WTD heads and it has been shown recently that PrP^{CWD} may not be bilaterally present in select tissues[32,35]. The 10 positive animals (originating from wild WTD herds) selected for testing of neck muscles were strongly positive across multiple tissues and likely were in relatively advanced, yet pre-clinical stages of the disease (i.e., no clinical signs were observed at the time of euthanasia)[32]. The stability of prions may vary depending on strains[36] and, to date, no RT-QuIC method is available to detect particular CWD strain differences. Further, the progression of CWD affects the deposition of prions in peripheral tissues and it is unclear at what time in the disease progression that prions accumulate in WTD muscles. We note the neck muscles used herein were frozen less than 12 h after collection; however, the other muscle tissues were at various stages of decomposition and underwent multiple freeze–thaw cycles prior to our possession. This difference in tissue preservation and quality potentially accounts for the reduced sensitivity of RT-QuIC upon application, an observation that suggests an altered balance of RT-QuIC inhibitors and active prion seeds and/or degradation of particular CWD prion strains in decaying tissue. Thus, we recommend muscles for RT-QuIC-based analyses of CWD prions be frozen (at either −20 °C or −80 °C) as soon as possible after collection, ideally less than 24 h.

Based on the results from well-preserved neck muscles, we posit that the freeze–thaw method has the most potential for large-scale diagnostic screening of venison, as it is cheaper and easier to perform. For samples with heavy prion loads, such as tongue, all methods used in this study agreed on the positivity of prion-seeding activity. For poorly preserved sample types, collagenase A outperformed the freeze–thaw method and trypsin digestion in terms of identifying more RT-QuIC positive muscle samples from CWD positive animals. Surprisingly, trypsin digestion yielded a high RAF and did not require additional dilutions of the final resuspension as needed by other methods. This could be due to the digestion of protein inhibitors by trypsin and/or superior ability of trypsin to free prions from examined tissues. Additional optimization of the methods presented here is needed for protocols focused on suboptimal sample types. It is possible that the prion seeding activity we detected in the collected muscle tissues is from non-muscle cell types as reported by Daus et al.[28]. However, the cellular origin of PrP^{CWD} in skeletal muscle, whether in myocytes, erythrocytes, neurons, epithelial cells, or any other cell type, is inconsequential to the recommendations of not consuming venison from CWD-positive animals or the potential for RT-QuIC-based venison screening as venison products are a matrix of multiple tissues and cell types.

Our findings suggest that CWD prions occur throughout an array of WTD muscles and further investigation, from an anatomical perspective, is needed to understand the extent of this distribution. Future studies focusing on larger sample sizes with systematic, bilateral samplings of well-preserved muscle samples throughout the body are needed to assess, validate, and improve the presented method for its application, as well as quantify the load of CWD prions present. Longitudinal characterization of prion deposition (i.e., using cervid challenge experiments) in a variety of high-quality muscle samples, such as those conducted for saliva, lymphoid tissues, and feces is needed to better understand the pathophysiology of CWD in deer and other cervids. Our study provides the foundation for the development of RT-QuIC-based screening of venison and venison-related products associated with food processing pipelines for CWD-prions.

Methods

Experimental design. The RT-QuIC muscle protocols (freeze–thaw and enzymatic digestion, detailed below) were initially used on a small subset of CWD positive and not-detected neck muscle tissue samples. After refining our methods, we then tested the protocol on a larger set of neck muscles from ten CWD positive and ten CWD not detected deer, with CWD status determined by ELISA, IHC, and RT-QuIC analyses on lymphoid

tissues from the animals reported by Schwabenlander et al.[32]. To blind investigators, researcher "A" subsampled approximately 300 mg of each sample, placed them individually in 1.5 ml tubes, and re-labeled them in a randomized numerical order. Researcher "B" carried out the muscle processing and RT-QuIC and was blinded to the original identity of the samples. ELISA and IHC results for animals examined herein are presented in Supplementary Table 1. Researcher "B" was unblinded after the first pass of all samples; investigation of different extraction methods was done after unblinding.

Sample collection. RT-QuIC protocol development was initially performed utilizing neck muscle (*brachio cephalicus*/*sternocephalicus*) tissue samples collected from wild WTD through 2019 agency culling operations in southeast Minnesota conducted by the Minnesota Department of Natural Resources in conjunction with USDA APHIS Wildlife Services as described by Schwabenlander et al.[32] (Table 1). Muscle tissue samples for the quantitative comparison study were obtained through disposal or necropsy of farmed and wild WTD at the University of Minnesota Veterinary Diagnostic Laboratory and were stored at − 20 °C. All farmed and wild WTD examined in our study have been independently tested through official regulatory means by the National Veterinary Services Laboratories or Colorado State University, respectively, for CWD infection based on immunodetection analysis (ELISA and/or IHC) of the brain and/or lymphatic tissue for the presence of PrP^{CWD} (Table 2).

Muscle preparation. *Freeze–thaw method.* This method was inspired by a combination of existing RT-QuIC protocols[20,25,28]. Muscles were stored at − 20 °C within 12 h after collection then transferred to − 80 °C until tested. 10% (weight/volume) muscle homogenates in 1× PBS were prepared in tubes with 1.5 mm diameter zirconium oxide beads using a Beadbug homogenizer at top speed for 180 s. The homogenates underwent three cycles of flash freeze–thaw consisting of 3 min in dry ice and 3 min at 37 °C. The homogenates were subjected to additional homogenization at top speed for 180 s using the Beadbug homogenizer. The mixtures were then centrifuged at 5000 rpm for 3 min. 500 µl of supernatants were used for centrifugation at 15,000 rpm, 4 °C for 40 min. The resultant pellets were resuspended in 100 µl of 1× PBS then incubated with 7 µl of 4% (w/v) phosphotungstic acid (Sigma-Aldrich) in 0.2 M magnesium chloride. The mixtures were then incubated at 37 °C and 1500 rpm for 1 h in a ThermoMixer (Eppendorf) before being subjected to centrifugation for 30 min at 15,000 rpm, 4 °C. Pellets were resuspended in 10 µl of 0.1% (v/v) SDS/PBS/N2. 2 µl of 10-1 diluted resuspension was used for optimal result.

Collagenase A and trypsin digestion method. This method for RT-QuIC was modified from the PMCA method developed by Daus et al.[28]. 10% (weight/volume, w/v) muscle homogenates and 180-s homogenization were carried out as described above. 350 µl homogenates were mixed with equal volume of 2× collagenase A [4 mM $CaCl_2$ and 0.5% (w/v), Roche] or trypsin (Gibco) solutions. The mixture was incubated at 37 °C, 700 rpm for four hours. After being homogenized again for 90 s, the mixtures were centrifuged at 5000 rpm for 3 min at 4 °C. The supernatant was then transferred to another tube and mixed with an equal volume of 2× protease inhibitor cocktail (Sigma-Aldrich). This was followed by steps including centrifugation at 15,000 rpm for 40 min as the freeze–thaw method. 2 µl of the final suspensions were diluted tenfold and added to the RT-QuIC reaction. The additional dilution was not necessary for trypsin digestion.

RT-QuIC substrate preparation and reaction conditions. Recombinant hamster PrP (HaPrP90-231; provided by NIH Rocky Mountain Laboratory) production and filtration followed the methods of Schwabenlander et al.[32]. All ingredients of RT-QuIC master mix (1× PBS, 500 µM EDTA, 50 µM ThT, 300 mM NaCl, and 0.1 mg/ml HaPrPrP) were filter-sterilized through 0.22 µm PVDF filters. 98 µL of the master mix was pipetted into wells of 96-well black clear bottoms plates. The plate was sealed with clear tape after 2 µL samples were added. Plates were then shaken on BMG FLUOstar Omega microplate readers (BMG LABTECH Inc., Cary, North Carolina, USA) at 700 rpm (57 s double orbital shaking followed by 83 s resting). Fluorescence was recorded after 21 shake/rest cycles using a 450 nm excitation filter and 480 nm emission filter. The gain was set to 1600. The machine performed 21 flashes/well and no well-scan was conducted. 45 °C, 50 °C, and 55 °C were used. 55 °C was only used for investigating whether decomposing tissues would have converging dilutions. 50 °C was used for enzymatic digestions.

Data analysis. Statistical analysis and plotting of fluorescence data from RT-QuIC were conducted using GraphPad Prism version 9.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. RT-QuIC data from four or eight replicates were used for calculating the rate of amyloid formation (RAF) for muscles, which is defined by the inverse of the time to reach the fluorescent threshold[15]. The threshold was calculated as ten standard deviations above the average baseline fluorescence unless otherwise specified. We observed variable RAF values across the four microplate readers used for our analyses (i.e. plate reader one consistently exhibited earlier amyloid seeding rates vs. plate readers two, three, and four). In no instance did this impact our positive or negative controls. However, due to RAF differences among plate readers, this threshold could not be applied to all plates. In these rare circumstances, the threshold was calculated as two times the background fluorescence in each well. The differences in RAF calculated by these two methods for a true RT-QuIC positive sample is usually less than 0.01, therefore not influencing general comparisons of RAF among plates. The one-tailed Mann–Whitney unpaired u-test was used to test the average difference between samples and corresponding negative controls on the same plates. Quantitative analysis of CWD prion load in muscles was conducted as described by Henderson et al.[15].

Animal research statement. No white-tailed deer were euthanized specifically for the research conducted herein and all tissues were secured from dead animals or loaned for RT-QuIC analyses. For these reasons, the research activities conducted herein are exempt from review and approval by the University of Minnesota Institutional Animal Care and Use Committee (as specified https://research.umn.edu/units/iacuc/submit-maintain-protocols/overview). All CWD positive deer were submitted to the University of Minnesota College of Veterinary Medicine for disposal of infectious prions and were sampled prior to their disposal. White-tailed deer were euthanized by the Minnesota Department of Natural Resources (MN DNR) for annual culling efforts to control the spread of CWD in Minnesota following MN DNR state regulations and euthanasia guidelines established by the Animal Care and Use Committee of the American Society of Mammalogists[37]. All methods and all experimental procedures carried out during the course of this research followed University of Minnesota guidelines and regulations as approved by the Institutional Biosafety Committee under protocol #1912-37662H. This study was also carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org). We confirm that no human tissues were used for the research performed herein.

Field research. CWD negative animals were euthanized by the Minnesota Department of Natural Resources for routine annual culling efforts to control the spread of CWD in Minnesota and were not sampled specifically for the current study. Tissue samples were provided by the MN DNR.

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Author contributions

M.L., M.D.S., D.S., and P.A.L. conceived of the study. M.L. designed the RT-QuIC experiments, collected data, and performed data analysis. M.L. and G.R.R. interpreted RT-QuIC results and prepared figures. M.L., M.D.S., D.S., and P.A.L. wrote the main manuscript. G.R.R., J.M.S., C.S.J., and M.C. helped to interpret results and write the manuscript. All authors reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

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THE WILDLIFE PROFESSIONAL

Hitting the Lights

Illuminating the effects of artificial light at night

The 'N of 1 problem' in management agencies

A complex disease, simplified

Combating climate change with 'poo-namis'

THE WILDLIFE SOCIETY

A Complex Disease, Simplified

INNOVATIVE TOOLS HELP PRESENT CHRONIC WASTING DISEASE EDUCATION TO DIVERSE AUDIENCES

By Marc D. Schwabenlander, Anna E. Pendleton, Tiffany M. Wolf, Peter A. Larsen and Roxanne J. Larsen

In February 2019, a team from the University of Minnesota appeared before state legislators to talk about chronic wasting disease. Speaking at an informational hearing before the Minnesota House Environment and Natural Resources Finance Division, assistant professor Peter Larsen and his team planned to focus on a multiyear research plan to develop cutting-edge diagnostic tools to combat the disease. But they faced a curveball from state Rep. Jean Wagenius. With CWD becoming a growing concern in Minnesota, Wagenius wanted to know what the university planned to do — *immediately* — to educate the public.

The concerns did not end with the hearing. Later in the year, as deer hunting season drew to a close, state legislators and agency leaders called Larsen to talk about the lack of CWD information reaching hunters. They were particularly concerned about a lack of information reaching underserved communities — specifically the Amish, Hmong and Tribal communities. Additional communications with stakeholders and partners made it clear that the needs were even broader. A vast spectrum of the public —K-12 students; graduate students; civic leaders; lawmakers; agency personnel; landowners; deer, elk and caribou farmers; subsistence hunters; recreational hunters — all needed more CWD education.

It was clear that Minnesota's CWD efforts needed to go beyond diagnostics.

Credit: Peter Larsen

▼ **Tiffany Wolf explains CWD through images and an animation of CWD infection in a deer's body to a group of elementary school students at a February 2020 Science Museum of Minnesota public event.**

Preparing the way

CWD is a contagious, neurodegenerative disease affecting wild and farmed cervids, and it is 100% fatal. In the family of transmissible spongiform encephalopathies, it is caused by malformed prions or proteins that become infectious when misfolded. The disease was originally described in mule deer (*Odocoileus hemionus*) in Colorado in 1967, but since the mid-1990s, its occurrence rate and geographic spread have dramatically increased across the United States and Canada (Rivera et al. 2019). Sometimes dubbed the "zombie deer disease," its spread, persistence and potential for cross-species transmission make it worthy of a Stephen King novel.

CWD is a serious threat to deer heritage, raising concerns about its impacts on recreational and subsistence hunting, cultural ceremonies and wildlife viewing. It is also a threat to economies, posing risks to cervid farming on a national level to state natural resources income to local meat processors, taxidermists and forage crops. The risks have spread across North America to parts of northern Europe. Yet, much of the fear that surrounds it — and ironically, much of the apathy, too — comes from general misunderstanding of CWD and the basics of prion diseases.

Responding to state leaders' concerns, Larsen recruited a team to address CWD educational needs.

These grassroots efforts were the first steps in organizing the Minnesota Center for Prion Research and Outreach, or MNPRO. The multidisciplinary center would focus on the biology and epidemiology of human and animal prion diseases and related protein-misfolding disorders, including CWD. The center developed materials to complement resources provided by the Minnesota Board of Animal Health and the Minnesota Department of Natural Resources, which mostly dealt with regulations.

The collaborative approach was intended to result in clear and consistent messaging that supported state policies and regulations and met diverse informational needs of stakeholders. It was a critical goal, since stakeholder behavior can impact CWD management (Vaske 2010). MNPRO focused on developing and disseminating education materials to those affected by CWD, both inside and outside endemic regions of the state. The materials specifically targeted Amish, Hmong and Tribal communities, which are underrepresented and underserved in the realms of natural resources and wildlife disease management and education.

Taking action

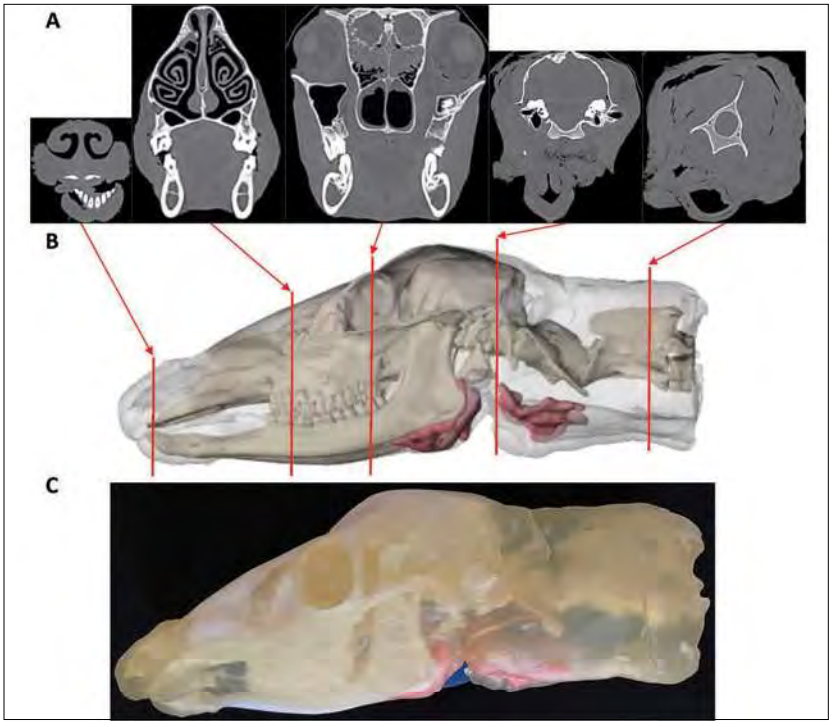
Partnering with the university's Center for Animal Health and Food Safety, we were able to create a diverse, dynamic set of educational resources. By utilizing the networks of a land grant university system, we were able to reach a wide variety of audiences through direct interaction. Keeping politically neutral, we used common language to offer fair and impartial treatment and meet stakeholders at their level of knowledge. Working closely with Amish, Hmong and Tribal community representatives, we made sure we included community and cultural values, beliefs and norms in our materials while following best practices in science communication and outreach — all key elements in becoming a trusted source of CWD information (Vaske 2010; Vaske and Miller 2018).

Scientists and educators often use innovative visualizations — from simple diagrams to virtual reality — to communicate complex scientific concepts across disciplines, whether it be ecology (Schtickzelle et al. 2020), molecular and cellular biology (Jenkinson 2018), anatomy (Fredieu et al. 2015), engineering (Ford & Minshall 2019) or geology (Cho & Clary 2020). Our CWD educational materials followed a natural progression of engagement and technology, becoming increasingly complex as our efforts continued.

Credit: Marc Schwabenlander

◀ **Peter Larsen explains the physical structure of an abnormal, infectious prion protein using a Slinky at an October 2019 Winona State University public event.**

Our public outreach included in-person presentations and printed materials that described the heritage of deer in North America, discussed how CWD spread into Minnesota and presented some of the efforts scientists were undertaking to test, diagnose and research the disease. One-page fact sheets written in English were translated into several other languages, including Hmong, Khmer and



Credit: Roxanne Larsen

▼ **This visualization shows the steps in creating a 3D-printed deer head and neck for CWD sample collection training. A) A deer head and neck CT scan. B) A digitally constructed model. C) A 3D-printed model.**

Korean. A booklet created for Minnesota's Amish communities was modified for use in K-12 education and translated into Hmong, incorporating ideas and imagery describing basic CWD biology.

Knowing that analogies, metaphors and visualizations can help convey complex science to broad audiences (Jenkinson 2018), we prioritized using visual aids of familiar objects. Using Slinky toys, we illustrated how prions function and helped broad audiences grasp a topic as abstract as prion disease. We used an ordinary Slinky to represent a normal prion. Twisted, bent or stretched Slinkies showed how prions can be misfolded or damaged.

Credit: Carolyn Bernhardt

▼ **Peter Larsen demonstrates the 3D deer head model to graduate students Manci Li and Peter Christenson prior to collecting samples for CWD testing in March 2021.**

Since videos and animations can help tell a story with motion (Jenkinson 2018), our team developed and assembled a variety of informational videos and recordings of public presentations to create a video library on topics including basic CWD biology and the science behind testing, as well as basic guides for hunters harvesting deer. An animation was designed to illustrate CWD transmission between deer and show how CWD prions may affect a deer's body, detailing the process of how they are ingested and pass through the body. The animation inspired the booklet design and has been used in public presentations to further engage attendees.

As 3D printing has become less expensive and more available, it has emerged as a method used at all levels of education (Jenkinson 2018; Ford & Minshall 2019). We developed 3D models of a white-tailed deer (*Odocoileus virginianus*) head and neck to assist in training individuals on tissue sampling for CWD testing. To create the model, a CT-scanned deer specimen was digitally segmented, and components of the anatomy were 3D-printed in a single model. We also created short video clips detailing the process of lymph node identification and removal for CWD testing. The model and associated materials are used to educate hunters, cervid farmers, veterinary students, wildlife biologists and others on locating and identifying the medial retropharyngeal lymph nodes, a necessary advancement as the need for sampling increases and fewer funds are available for professional samplers.

Augmented reality is relatively new, but it is increasingly being used in a variety of educational settings (Jenkinson 2018). We used the platform Zappar to create an interactive display featuring augmented reality. The display presented the image of a healthy deer that, when viewed through a smartphone or tablet, transitioned to a sick deer showing clinical signs of CWD. Combining 2D and 3D visualizations, it allowed participants to learn independently at larger public outreach events.

Many of our resources have been made available electronically through CWDWatch, Hunters' Toolkit and MNPRO websites and social media platforms, particularly as in-person outreach diminished with the COVID-19 pandemic. To increase access, our educational materials were developed for many platforms: print, broadcast and social media; websites; webinars; seminars; in-person presentations; museums; even testimonies at legislative sessions. Known as "just-in-time learning," the approach allows those who need the information at a given time to have accessible, relevant resources available. These tools helped us reach communities most impacted by CWD, such as farmers with CWD-positive cervid herds and hunters and community members in areas with CWD-positive wild deer or where CWD surveillance is performed.

On the right path

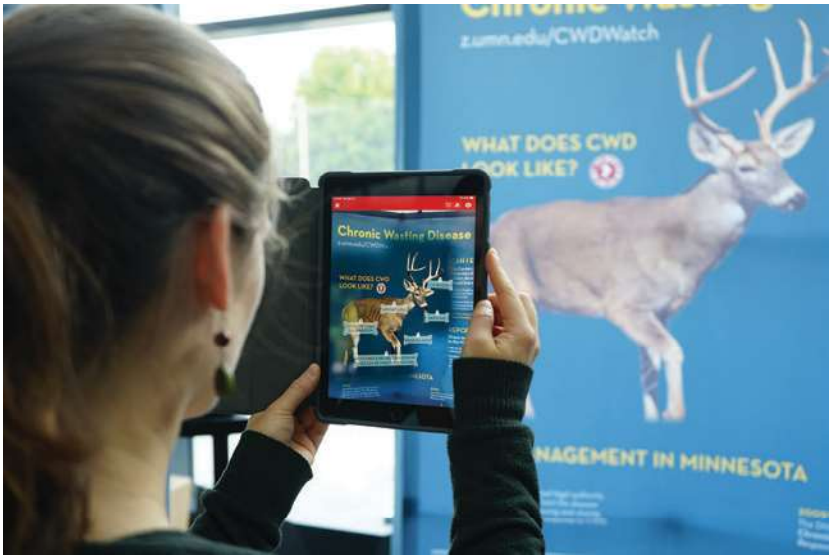
Since developing these resources, our team has interacted with a range of audiences. K-12 students and graduate students. English speakers and those who speak or read other languages. Civic leaders and lawmakers. Regulatory and management agency personnel. Landowners and cervid farmers. Subsistence and recreational hunting communities.

From late 2019 to early 2021, our group presented at over 30 in-person and virtual public events, reaching more than 6,000 people. Based on discussions generated at these sessions, audience engagement was clear and the informational resources were welcomed. Stakeholders provided positive and constructive feedback on the comprehensible nature of our material and their enhanced awareness of CWD. After participating in a public event at the Science Museum of Minnesota, museum leadership affirmed that the face-to-face interaction between scientists and the public was a much needed but rare form of engagement. Our website metrics indicate 2,430 unique page views, and our videos have collectively been viewed over 3,000 times, extending the educational reach beyond in-person events and outside Minnesota's borders.

While these educational materials were informed and appreciated by our stakeholders, future work is needed to fully assess the impact of our efforts and those of our colleagues. MNPRO's future CWD outreach materials will be further developed and influenced by information directly gathered from affected communities through a community-based participatory research initiative, which has incorporated Hmong and Tribal community members as research and education partners.

Incorporating assessment tools, such as surveying audiences before and after materials have been used, are considered best practices for measuring the usefulness and effectiveness of educational materials (Vaske 2010). By incorporating assessment models that infer the ability to change attitudes and behaviors through educational engagement, outreach programs can respond with the most effective educational and communication tools to best fit stakeholder needs and improve CWD management.

Future educational and outreach initiatives will also need to involve the coordination of collaborators, as exemplified by the multidisciplinary members and objectives of the USDA-funded NC1209: North American Interdisciplinary Chronic Wasting Disease Research Consortium, poised to advance CWD research, including CWD outreach and education. Although there is mutual interest among governmental, tribal, university, nonprofit and other entities in providing stakeholders with up-to-date CWD resources, we all face challenges in material development and distribution.



Credit: Anna Pendleton

▲ **An attendee at a September 2019 Bell Museum of Natural History public event investigates augmented reality showing the image of a healthy deer that, when viewed through the tablet's Zappar App, transitions to display a sick deer showing clinical signs of CWD.**

Representative Wagenius's heartfelt inquiry opened our eyes to CWD educational needs in Minnesota, and we are now better prepared. As these tools and partnerships continue to advance, we hope others can use the information in their own CWD outreach. In the end, sharing validated resources and best practices across all groups engaged in CWD outreach and education will lead to consistent messaging more likely to reach broader audiences and achieve greater impact in CWD management. It is time to travel down this road collectively, constructively and candidly. ■

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VETERINARY RESEARCH

REVIEW Open Access

Chronic wasting disease: a cervid prion infection looming to spillover

Alicia Otero[1,2,4], Camilo Duque Velásquez[1,2], Judd Aiken[2,3] and Debbie McKenzie[1,2*]

Abstract

The spread of chronic wasting disease (CWD) during the last six decades has resulted in cervid populations of North America where CWD has become enzootic. This insidious disease has also been reported in wild and captive cervids from other continents, threatening ecosystems, livestock and public health. These CWD "hot zones" are particularly complex given the interplay between cervid *PRNP* genetics, the infection biology, the strain diversity of infectious prions and the long-term environmental persistence of infectivity, which hinder eradication efforts. Here, we review different aspects of CWD including transmission mechanisms, pathogenesis, epidemiology and assessment of interspecies infection. Further understanding of these aspects could help identify "control points" that could help reduce exposure for humans and livestock and decrease CWD spread between cervids.

Keywords: Chronic wasting disease, prion, prion disease, prion pathogenesis, interspecies transmission

Table of Contents

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1 Introduction

Chronic wasting disease (CWD) is a highly prevalent prion disease affecting various species of the *Cervidae* family and has been described in North America, South Korea and Scandinavia [1, 2]. Prion diseases are fatal neurodegenerative disorders affecting numerous mammalian species. In addition to CWD, prion diseases include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy (TME), and Creutzfeldt-Jakob disease (CJD) in humans. CWD is the only prion disease affecting both wild and farmed animals and stands out for being highly contagious, widespread and persistent in the environment, which facilitates the transmission of the disease and hinders its control in deer populations [3–6].

Pathogenesis of CWD, as described for other prion diseases, occurs over extended asymptomatic periods and depends on the misfolding of the cellular prion protein (PrP^{C}), encoded by the *PRNP* gene, into an infectious template-directing conformation (PrP^{Sc}) [7]. Following exposure to prions, the host's susceptibility to develop disease, the clinical presentation, and the neuropathology are regulated by the interaction between the host PrP^{C}

primary structure and the invading prion agent or strain [8–10].

The neuropathology of affected cervids includes spongiform degeneration, neuronal loss, gliosis, and accumulation of PrP^{CWD} (cervid PrP^{Sc}) in the form of aggregates [3, 4, 11]. Variation in the disease presentation between cervids, including survival period, distribution of brain lesions and PrP^{CWD} properties occur in concert with different prion strains [12–15]. Prion strains are reproducible biological information encoded in specific PrP^{CWD} conformers that are replicated by the templated-misfolding of the host PrP^{C} [16–18]. While the transmission of a prion strain between hosts sharing similar PrP^{C} is more efficient given the compatibility of selected strain-specific PrP^{Sc} conformers, transmission between hosts species expressing different PrP^{C} primary structures is relatively inefficient and can introduce permanent conformational modifications resulting in the emergence of strains with novel properties [14, 19–21]. Alternatively, a strain can be transmitted back and forth between two species expressing various PrP^{C} amino acid differences and remain unaltered informationally [22–24]. Strain selection from a host co-infected with multiple strains can also occur following transmission between species expressing different PrP^{C} molecules [12, 25]. In addition, some tissues may show differential susceptibility for some strains compared to the brain (e.g. spleen) [26].

The transmission cycle of CWD in wild and captive cervids also involves the propagation of prion strains within and between various host species expressing distinct PrP^{C} primary structures [12, 27, 28]. As CWD outbreaks become enzootic in cervid populations, circulating CWD strains must adapt to the shifting PrP^{C} landscape that each new host provides which might result in novel strain emergence [13, 14, 21, 29]. These shifts in the prion replication substrate (PrP^{C}) also occur at the population level, with allele frequencies of protective PrP^{C} polymorphisms increasing in response to CWD in deer and elk [27, 30]. Here, we review the current understanding of the CWD transmission cycle, pathogenesis, infection biology and infer from numerous bioassay studies the potential for transmission to other species, including humans.

2 Transmission of CWD

The progression of CWD is less understood in wild free-ranging cervids given its direct relationship with the infectious dose, the route of exposure, the prion strain and the host *PRNP* genotype. Under controlled conditions, the incubation period (i.e., time to onset of clinical signs) of experimentally infected, orally dosed white-tailed deer, mule deer and reindeer expressing different PrP^{C} ranged between 1.5 and 6 years post-exposure [13, 31–33]. Similarly in elk, differences in incubation period were observed to range between 1.8 to 5.2 years depending on the elk *PRNP* genotype [34–36]. During the asymptomatic period, both captive and wild infected cervids contribute to the spread of CWD as they accumulate considerable amounts of prion infectivity throughout the body, which is shed through secretions and excretions into the environment [6, 37, 38].

The age range documented for cervids infected with CWD in captivity is fairly similar to that described for free-ranging animals [39, 40]. However, depending on the historical CWD prevalence and given the social nature of cervids it is possible to find pre-clinical CWD positive fawns (< 1 year-old animals) and yearlings (1–2 year old) [41]. The youngest identified clinically-positive free-ranging deer and elk were 16 and 21 months of age, respectively [4, 42]. An age range of 2.5–7.5 years has been reported for free ranging clinical deer and 1.8 to 10.5 years for elk [4]. More recently, evaluation of the prevalence of CWD infection by age as determined by detection of abnormal prion protein in 28 954 deer collected over seven years of surveillance in Wisconsin and Illinois enzootic zones reached similar conclusions as in the previous studies; the risk of CWD infection increases with age in both male and female deer (prevalence in adults 1.93%, yearlings 0.89 and 0.45% in fawns) [43]. Previous analyses of prevalence in a sample of 4510 deer culled within Wisconsin eradication zones, where CWD incidence has historically been and remains the highest, confirmed CWD infection in 3 to 3.4% of yearling deer irrespective of sex [44]. These findings suggest the higher prevalence in younger animals (yearlings and fawns) could be related to the extent to which CWD has been historically prevalent within a particular cervid population. The detection of prions in fawns could as well represent mother to offspring transmission [45].

Differences in age at which clinical CWD is observed can vary between species and are likely to depend on the source or origin of the infection. The first cases of CWD Scandinavian cervids, were described in various 9–16 year old moose, a 16 year-old red deer and 3–4 year old reindeer [2, 46, 47].

The prevalence of CWD in wild cervids also varies by sex. In CWD enzootic areas the incidence of infection is much higher in males than females [44, 48, 49]. Considering that no differences in susceptibility have been detected between captive male and female deer [3, 39, 40, 50, 51], the higher prevalence in free ranging males may be attributable to differences in behavior, particularly during the breeding season when males roam widely and interact with other males more avidly, increase the risk of contact with contaminated environments and infected animals [52, 53].

The clinical progression and signs of CWD in both captive and experimentally infected cervids vary within and between species [3, 13, 31]. A brief summary is included in Table 1. Initial clinical features are often subtle and transitory [13]. The most prominent clinical features include behavioral alterations and progressive deterioration of body condition (i.e., weight loss) that worsen over the course of weeks to months [54]. Altered postures with lowered head and ears, arching of the back and ataxia can also be displayed [13, 31, 42]. Advanced clinical disease may involve odontoprisis, polydipsia, polyuria, difficulty swallowing, regurgitation of rumen contents and excessive salivation with drooling. Following recumbency, aspiration pneumonia, dehydration, or hypothermia during the winter season (in wild affected animals) are the most likely causes of death [42]. Compared to deer, CWD-affected elk can present with nervousness and hyperesthesia and are more likely to display motor disturbances but less likely to develop polydipsia [54].

Various factors contribute to the efficiency of the CWD transmission cycle. The primary mode of transmission between cervids, early in an outbreak, is likely by direct animal to animal interactions following exposure to an infected animal or environment [53, 55]. Although experimental infection in pregnant muntjac deer (*Muntiacus reevesi*) and detection of PrP^{CWD} in tissues from wild pregnant elk dams provide evidence for in utero transmission [45, 56], other studies indicate it plays a minor role in epidemiology compared to deer-to-deer transmission [57]. Given the rapid pre-clinical accumulation of PrP^{CWD} aggregates in lymphoid tissues associated with the alimentary and the intestinal mucosa, the oral route of infection is most likely [31, 58, 59]. However, inhalation of CWD-fomites has also been proposed as a mechanism of exposure [60]. The presence of CWD infectivity in antler velvet [61] leads to the question of whether prions can persist in the calcified antler and favor male to male intradermal inoculation during the rut, or represent a risk factor in terms of oral CWD transmission, as antler gnawing is common among cervids. Antler gnawing has been suggested as a factor involved in the transmission of CWD in the reindeer population from Nordfjella, Norway [62].

The spread of CWD into "naïve" cervids also occurs through exposure to contaminated environments previously inhabited by infected animals [38, 57, 63]. This mode of transmission becomes more relevant as the prevalence of CWD in affected cervid populations increases and the disease becomes enzootic. Secretions (saliva) and excretions (urine and feces) of CWD-infected cervids contain considerable CWD infectivity. The minimum infectious dose in saliva required for a deer to become infected, assuming a single oral exposure to CWD prions, is equivalent to the infectivity contained in 100–300 ng of brain (approximate equivalent of 30 mL of CWD-positive saliva) [6]. Secretions and excretions from CWD positive animals and the decomposition of diseased carcasses contaminate the environment, in which prions can persist in a bioavailable state for years [38, 64–66]. The physical association of prions with certain soil microparticles enhances transmissibility [67, 68]. Environmental persistence of CWD infectivity depends on the composition of minerals and organic constituents of soil, which vary between geographical areas [69]. Minerals such as montmorillonite, can enhance the experimental transmission of prions by the oral route [67]. Deer consume a significant amount of soil, especially in adjacent areas to mineral licks, which have tested positive for infectivity in CWD endemic regions [70]. Soils, depending on their composition, represent an important reservoir of CWD infectivity in the environment. Although determination of the degree of contamination of a particular soil surface becomes more difficult with time, the soil-bound prion infectivity is not significantly altered [66].

Table 1 Summary of CWD presentation in different cervid species.

Cervid species	Average age range clinical disease	Clinical signs	PrP^{CWD} in the brain	PrP^{CWD} in lymphoid tissues	References
Mule deer White-tailed deer	2.5–7.5 years	Poor body condition, altered postures, ataxia, odontoprisis, polydipsia, polyuria, drooling	Yes	Yes	[42, 54]
Elk	1.8–10.5 years	Poor body condition, nervoussness, hyperesthesia, ataxia	Yes	Yes	[84]
				No	[83]
Reindeer	3–4 years	Poor body condition, recumbency, lethargy	Yes	Yes	[2]
Moose	9–16 years	Poor body condition, reduced fear of humans	Yes	No	[46]
Red deer (Minnesota)	22 months	Recumbency	Yes	Yes	[156]
Red deer (Norway)	16 years	No clinical signs observed	Yes	No	[47]

Plants can also represent an important reservoir for CWD contamination and transmission. CWD contaminated pastures can remain infectious for at least 2 years after prion exposure [65]. Regarding the uptake of prions by plants, results are more controversial. One study, using protein misfolding cyclic amplification (PMCA), an ultrasensitive technique for the detection of prions, demonstrated that grass plants exposed to brain or excretions from CWD-infected cervids can uptake prions from the soil and transport them to the aerial parts of the plant [71]. Another study, however, showed that wheat plants do not transport CWD prions from the roots to the stems [72].

3 Prion neuroinvasion and body distribution of infectivity

The pathological hallmarks of CWD in deer resemble those observed in sheep with scrapie and other prion diseases acquired by ingestion of contaminated material. In orally infected deer, PrP^{CWD} crosses the intestinal epithelial barrier and can be detected, within the first 30–42 days post-exposure, in lymphoid tissues associated with the alimentary tract such as the gut-associated lymphoid tissue (GALT), the tonsils and the retropharyngeal lymph nodes [31, 59, 73, 74]. Modified enterocytes, called M cells, participate in the uptake of the prion, incorporating it into the subepithelial lymphoid tissue. The pathological prion protein then accumulates and replicates in follicular dendritic cells and tingible body macrophages [58, 75, 76]. PrP^{CWD} cellular targeting during early pathogenesis suggests that prions are transported by dendritic cells and/or macrophages to Peyer's Patches and regional mesenteric lymph nodes [58, 59].

Once infection has been established in the GALT, prion colonization of the nerve endings of the Enteric Nervous System (ENS) and leakage into the lymph and blood facilitates the spread to other organs [77, 78]. Prion infection of the ENS results in the spread of infectivity through sympathetic and parasympathetic nerves [75]. The initial site of PrP^{CWD} detection within the deer brain is the dorsal motor nucleus of the vagus nerve (DMNV), suggesting this nerve as the major route for PrP^{CWD} traffic from the alimentary tract to the brain [73].

CWD infectivity trafficked via the lymph and the blood can reach multiple organs, including the brain. Prion neuroinvasion by this route likely occurs via the circumventricular organs [79]. Consistent with this observation, considerable prion infectivity has been demonstrated in numerous blood cell types from CWD-infected deer, suggesting that the haematogenous dissemination of infection may be important during the pathogenesis of the disease [80].

Another major route of prion neuroinvasion involving the entry via ENS is by retrograde transport of prions through the splanchnic nerve circuitry. This is consistent with the presence of prion aggregates in the intermediolateral columns of the thoracic spinal cord during early stages of prion infection [81, 82]. This route is particularly important during neuroinvasion by BSE prions in cattle, however, analysis of CWD prion accumulation following oral infection did not detect prion deposits in the coeliac ganglion of deer. This suggests that the accumulation of PrP^{CWD} in the intermediolateral column of orally infected cervids results from the centrifugal dissemination of prions replicated within the central nervous system (CNS) [73].

The early lymphoid replication phase is particularly important for the neuroinvasion of CWD prions in deer [73, 74]. Interestingly, North American elk as well as Scandinavian moose and red deer accumulate PrP^{CWD} in the brainstem with little to no accumulation in lymphoid tissues [46, 47, 83, 84]. This could be explained by a predominantly neural route of neuroinvasion (as in BSE pathogenesis), sporadic misfolding of PrP^{C} or differences in the route of exposure. Differences in pathogenesis and neuropathology of sheep inoculated via different routes were not seen [85]. Similarly, no significant differences have been detected in the peripheral burden of CWD prions from deer infected through different routes [64]. Once neuroinvasion has occurred, PrP^{CWD} accumulates producing the characteristic lesions of prion diseases, including intraneuronal vacuolation, neuropil spongiosis, gliosis and formation of amyloid plaques [86].

In addition to lymphoid and brain tissues, CWD prions have been detected in nasal mucosa, salivary glands, urinary bladder, pancreas, kidney, intestine and reproductive tract of female and male deer [15, 31, 64, 73, 87, 88]. The accumulation of PrP^{CWD} in some of these tissues is tightly associated with shedding of infectivity through secretions and excretions [64]. Similar to scrapie in sheep [89], *PRNP* genotype can influence CWD pathogenesis in deer affecting PrP^{CWD} deposition in peripheral tissues [13, 15, 31, 74, 90].

4 CWD in cervids

The origin of CWD remains unknown. In North America, epidemiological data suggests emergence occurred in Colorado and Wyoming [55], in the late 1960s, in captive mule deer (*Odocoileus hemionus*) and black-tailed deer (*Odocoileus hemionus columbianus*) at research facilities. These herds were captured cervids from different wild populations, including pregnant females that were released after parturition. Transfer of animals between facilities was a common practice [3]. CWD was subsequently detected in Rocky Mountain elk (*Cervus elaphus*

nelsoni) at these facilities and, thereafter, in free-ranging populations of mule deer and elk in Wyoming and Colorado [3, 50, 91].

Cervid migration and commercial movement of preclinical animals contributed to the geographic expansion of CWD into free-ranging and captive populations of North America [42, 55]. To date, CWD occurs in at least 26 U.S. states and three Canadian provinces (Saskatchewan, Alberta and Québec). In Canada, CWD was first identified in farmed elk from Saskatchewan in 1996 [42]. In the following years, CWD was reported in farmed white-tailed deer in Alberta and in wild cervid populations from Saskatchewan and Alberta [92]. Epidemiological studies suggested that the infection was introduced into Saskatchewan farms via import of captive elk from a farm in South Dakota [42]. The origin of the CWD epidemic in wild cervids of Canada remains unknown. Transmission by contact exposure between wild deer and infected farmed elk is a possibility [92]. CWD was first detected in 2013 in wild moose from Alberta [93]. CWD has not, to date, been detected in the wild North American subspecies of caribou (*Rangifer tarandus spp*).

Outside of North America, CWD outbreaks in captive cervids in South Korean farms occurred following cohabitation with asymptomatic infected elk and deer imported between 1994 and 2003 from a farm in Saskatchewan (later determined to house CWD-infected animals [94]. A direct consequence was the transmission into South Korean captive red deer (*Cervus elaphus*), sika deer (*Cervus nippon*) and crosses of these two species [28]. Epidemiological studies of CWD in wild cervids from the Korean peninsula are lacking.

In 2016, CWD was identified in a free-ranging Norwegian reindeer (*Rangifer tarandus tarandus*), representing the first CWD case detected in Europe [2]. Since this event, thousands of cervids have been surveyed, leading to the detection of the CWD in 19 reindeer, 4 moose and one red deer in Norway, 3 moose in Sweden and one moose in Finland, suggesting that CWD has been quietly emerging in European cervids [46, 47, 95]. The origin of these cases is still unknown. Transmission of Norwegian CWD isolates into bank voles demonstrated the presence of strains different than those seen in North America, suggesting these epizootics are not epidemiologically linked [96]. In addition, importation of cervids to Norway is not allowed and, therefore, it is unlikely that these CWD infections emerged from imported positive animals as was the case for CWD in Korea [1].

The prevalence of CWD in North America has been increasing exponentially during the last 6 decades. In farmed herds the prevalence of CWD positive animals can be higher than 80% and higher than 45% in wild populations [41]. In areas where CWD has become enzootic, the CWD prevalence can be greater than 50% in adult males [97]. The latest Alberta CWD surveillance update (2019 fall hunting season) indicates that the prevalence of CWD continues to increase in all cervid species. Compared to the 2018 fall hunting season, the prevalence in the 2019 season saw an increase of 3.8% (from 7.4% in 2018 to 11.2% 2019 prevalence). Consistent with previous years, white-tailed and mule deer in Alberta and Saskatchewan show differences in prevalence between species and sexes. The prevalence rank among Alberta deer is mule deer males > mule deer females > white-tailed males > white-tailed females. The burden of CWD in Alberta wild elk has not been as extensive as in deer. In 2019, 1.3% of the tested elk resulted positive for CWD (0.8% in 2018). In addition, for the first time, CWD was detected in two hunter-harvested moose [98].

Population declines are observed in cervid herds with high CWD prevalence. CWD positive deer not only succumb to the disease but are also more prone to be killed by predators or hunters, and are more vulnerable to vehicle collisions [99]. Average declines in elk survival in Rocky Mountain National Park were attributed almost entirely to CWD [100] Mean annual survival rates of CWD-negative and CWD-positive deer were estimated as 76% and 32%, respectively, and CWD was considered a significant contributor to mule deer population decline [101]. Miller et al. also observed that the 2-year survival of infected and uninfected tagged wild mule deer was 47 and 82%, respectively [99].

5 Experimental CWD in cervid species

No natural cases of CWD have been described in some species of cervids, although they have proven to be susceptible to CWD following experimental exposure. These include the Asian muntjac (*Muntiacus reevesi*) [45] and fallow deer (*Dama dama*) [102]. Muntjac deer were successfully infected through oral and subcutaneous routes with CWD from white-tailed deer. Interestingly, PrP^{CWD} was detected in fetuses from CWD-infected does, demonstrating vertical CWD transmission in this species [45]. Although fallow deer were suggested to show certain resistance to infection with CWD [103], Hamir et al. reported that this species is susceptible to the disease after intracerebral inoculation with elk and white-tailed deer prions [102]. The differences in these studies may have arisen because intracranial inoculation is more efficient to produce disease compared to environmental exposure, however, PrP^{C} sequence and prion strain compatibility could also explain these differences [102, 103]. No natural cases of CWD have been reported in North American caribou, although these species are susceptible to experimental infection with CWD from mule deer, white-tailed deer and elk. Naive caribou can acquire the

disease after oral infection [33] and environmental exposure [63]. A summary of this transmission experiments and the ones described below is included in Table 2.

6 Evaluating the potential transmission of CWD to non-cervid species

Most of the transmission studies of CWD into various animal species have been conducted with North American CWD isolates and have revealed different transmission patterns. The host range of European CWD isolates is still to be determined [95].

6.1 Livestock species

The interactions between different animal species in captivity is a known factor favoring the emergence of new pathogens with novel zoonotic properties, as has been recently proposed as the origin of BSE in cattle by contact with sheep infected with atypical/Nor98 scrapie [104]. The distribution of CWD in North America could favor the interspecies transmission of cervid prions into cattle (i.e., overlapping of these species is common in CWD enzootic areas of North America). The transmission of a prion disease to the cattle is cause for alarm due to the potential emergence of BSE-like zoonotic capacity. CWD from different species (white-tailed deer, mule deer and elk) has been successfully transmitted to domestic cattle after intracerebral challenge with different attack rates [105–108]. The neuropathological and biochemical characteristics of bovine CWD are, however, clearly distinct from BSE [105]. In addition, oral infections in cattle with mule deer prions have been unsuccessful, and no positive transmission has been detected in this species after 10 years of environmental exposure to mule deer and elk CWD [109]. This demonstrates that an important species barrier limits the oral transmission of CWD to cattle.

In 2006, Hamir et al. reported the transmission of mule deer CWD into sheep via the intracranial route [110]. Only 2 of 8 inoculated lambs developed lesions compatible with a prion disease, and they expressed different *PRNP* genotypes at codons 136, 154 and 171, which are known to determine sheep susceptibility to scrapie [111–113]. One animal expressed ARQ/ARQ (subclinical) and one ARQ/VRQ (clinical) sheep. ARQ/ARR sheep were completely resistant to CWD inoculation, suggesting that the transmission of CWD to small ruminants is strongly determined by the host genotype, as seen with scrapie [110]. Clinical disease was described, however, in ARQ/ARQ sheep inoculated with elk CWD prions, which suggests a different strain in the elk isolate. Transgenic mice overexpressing the ovine VRQ PrP allele (tg338 mice) do not accumulate prions in the brain after experimental infection with a number of different CWD isolates [26, 114, 115]. These tg mice, however, efficiently replicate CWD prions in the spleen, suggesting that the lymphoid tissue is more permissive than the brain for interspecies transmission [26].

The susceptibility of pigs to CWD has also been investigated. Moore et al. found that white-tailed deer CWD prions can be detected by real-time quaking-induced conversion (RT-QuIC) in some orally and intracranially inoculated pigs when euthanized at market weight (8 months age, 6 months after inoculation). One aged pig showed clinical signs and, in these aged animals, PrP^{CWD} was detectable by immunohistochemistry and Western blotting in 4/10 intracranially inoculated and in 1/10 orally inoculated pigs. Passages in transgenic mice expressing the porcine PrP showed reduced attack rates. Therefore, they concluded that pigs could support a low-level propagation of CWD prions, albeit with a high species barrier. These results are not necessarily encouraging since it is possible that feral pigs, whose ranges are shared with CWD affected cervids, could act as a reservoir of CWD [116].

Table 2 Experimental transmission of CWD to different animal species.

Species	Route of CWD transmission[a]	References
Cervids		
Muntjac deer	IC, PO, SC	[45]
Fallow deer	IC	[102]
North American caribou	PO, environmental	[33, 63]
Livestock		
Cattle	IC	[105, 108]
Sheep	IC	[110, 157]
Pigs	IC, PO	[116]
Wildlife		
Rodents		
Present in CWD endemic areas: Meadow voles Red-backed voles White-footed mice Deer mice House mice	IC	[29, 118]
Not present in CWD areas: Syrian golden hamster European bank vole	IC	[119, 120]
Carnivores		
Ferrets	IC, PO, IP	[121–123]
Mink	IC	[124]
Cats	IC, PO	[125]
Raccoons	IC	[128]

[a] IC: intracerebral, PO: oral, SC: subcutaneous, IP: intraperitoneal.

6.2 Other wildlife species

Comparison of the *PRNP* sequences of different species of ungulates that inhabit CWD endemic areas showed high sequence identity between bighorn sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus*) and domestic sheep suggesting that these species are potentially susceptible to CWD [117]. No experimental challenges of these wildlife species, sympatric to deer in CWD-endemic areas have been performed yet.

6.2.1 Rodents

Several different rodent species sympatric with deer in CWD endemic areas including meadow voles (*Microtus pennsylvanicus*), red-backed voles (*Myodes gapperi*), white-footed mice (*Peromyscus leucopus*) and deer mice (*P. maniculatus*) have proven to be susceptible to CWD after experimental inoculation [118]. Among these species, meadow voles showed to be the most susceptible, but incubation periods were shortened in all the rodent species upon second passage, indicating CWD adaptation to these hosts. House mice (*Mus musculus*) live in close proximity to humans, and their susceptibility to particular CWD strains has been demonstrated [29]. It is possible that wild rodents represent a reservoir for CWD in ecosystems considering that these animals are scavengers, and one of the main sources of food for predators. In addition, they can be accidentally consumed by deer or livestock since rodent carcasses contaminate pastures and forage [118].

CWD is also transmissible to other rodent species that do not cohabitate with deer in CWD-affected regions. These include Syrian golden hamsters (*Mesocricetus auratus*) [119] and European bank voles (*Myodes glareolus*) [120]. Curiously, the adaptation of CWD to the European bank vole resulted in the identification of a prion strain (CWD-vole strain) with the shortest incubation period observed to date [120].

6.2.2 Carnivores

Ferrets (*Mustela putorius*) are a valuable model for the study of prions, including CWD [121–123]. Mink (*Mustela vison*) can also be infected with CWD, but only by intracerebral inoculation. The disease characteristics differed from those of TME-affected mink, demonstrating different strains cause CWD and TME, and suggest that mink are unlikely involved in natural CWD transmission [124].

Oral and intracerebral inoculation of mule deer prions into domestic cats (*Felis catus*) resulted in no clinical disease or low attack rates, respectively, on first passage. A second passage of the prions from the intracerebrally inoculated cats resulted in 100% of the recipient cats presenting with clinical disease while the second oral passage resulted in a 50% attack rate demonstrating the adaptability of CWD prions to felines [125]. The PrP^C sequence similarity between cats and mountain lions (*Puma concolor*) suggests that these wild carnivores would be susceptible to CWD infection [126]. As mountain lions selectively prey CWD-infected cervids, it would be of interest to test dead animals for CWD to evaluate for prion spillover [127].

CWD prions from white-tailed deer and elk transmitted with low attack rates (25%) following intracerebral challenge in raccoons (*Procyon lotor*) [128]. Accumulation of protease resistant prions in the cerebrum and obex differed depending on the inoculum. Interestingly, prions from mule deer did not transmit to raccoons after 6 years following intracerebral challenge [129, 130]. This further suggests strain differences in CWD prions from the various cervid species affected.

Among all mammals, canids are probably the most resistant to prion diseases, with the amino acid residue 163 of canine PrP^C conferring protection [131–133]. Oral exposure of captive coyotes (*Canis latrans*) to elk prions demonstrated the presence of prions in the coyote fecal material during the first days after consumption [134]. However, even after a large volume of infectious brain homogenate was inoculated, only 50% of exposed coyotes had detectable infectivity in feces between 1- and 4-days post-exposure (dpe) as evaluated by bioassay in tg12 mice (expressing elk PrP^C), while the other half lacked detectable prions or were only recovered in feces after 1 day. No evidence of CWD accumulation in the coyote lymph tissue was detected [134]. These results suggest that coyotes were capable of degrading the CWD infectivity. Consistent with this interpretation, the attack rates were incomplete in tg12 mice inoculated with feces collected at various times following exposure. When inoculated with brain homogenates from CWD-infected elk, this transgenic mouse line develops prion disease with full attack rates after incubation periods of < 150 days post-infection [135]. In addition, in the wild, coyotes will likely consume a smaller infective dose as CWD infectivity in muscle and fat is lower than in the brain [51, 136]. The role of canine predators in the control of CWD has been discussed previously, suggesting that the selective predation exerted by wolves (*Canis lupus*), which hunt weak and vulnerable cervids, could represent an important natural tool to limit CWD contamination of the environment [137]. The reintroduction and protection of wolves in CWD-affected areas, although controversial, could be very efficient for the natural control of the disease.

6.3 Humans

To date, there is no clear evidence that CWD can cross the transmission barrier and infect humans, as other

animal prions such as BSE [138]. Several epidemiological studies have been developed to assess whether, statistically, there are more cases of prion diseases in population groups living in endemic areas for CWD. These studies mainly consider people exposed to CWD-infected cervids, such as consumers of deer meat and hunters. None of these studies have found a clear correlation between CWD exposure and an increase in human prion disease frequency [90, 139–141]. Evaluating the risk of humans to CWD through this type of studies is difficult due to the variety of strains present in the environment, the transport of hunted animals between long distances and the long incubation period of prion diseases in humans (even decades).The identification of the zoonotic ability of an agent requires an abnormally high number of human cases within a particular geographical location or period of time, which necessitates a large number of human exposures to the disease. Prevalence of CWD in several areas has increased exponentially in the last decade, therefore, there may not historically have been a sufficient level of exposure to the disease to detect a zoonotic transmission of CWD.

There are tools, however, to evaluate the susceptibility of humans to CWD. These include bioassays in non-human primates and transgenic mice expressing human PrP^C and in vitro studies of the human transmission barrier to CWD.

Squirrel monkeys (*Saimiri sciureus*) are susceptible to CWD prions from mule deer, elk and white-tailed deer after oral and intracerebral challenge [142, 143]. Race et al. did not observe evidence for CWD transmission to macaques (*Macaca fascicularis*) at 13 years post-inoculation and using ultra-sensitive techniques for the detection of prions [144]. In an ongoing study, macaques were exposed to different sources of CWD through various routes. Analysis of the tissues identified PrP deposition in the dorsal horns of the spinal cord in a subset of the macaques [145]. Similar PrP immunopositive staining affecting the spinal cord was also reported by Race et al.; these deposits were found in both CWD-challenged and uninoculated, aged macaques, suggesting that this staining was likely due to cellular PrP [144]. Evaluating the zoonotic potential to humans through bioassays in non-human primates has, however, several drawbacks. The degree of sequence similarity between human PrP and the PrP from non-human primates varies between 92.2 and 99.7% [146]. Even species with high sequence homology, such as chimpanzees, express amino acid substitutions in key structural motifs of PrP that could alter the transmission barrier of prions [146, 147]. Although chimpanzees are more closely related to humans, the presence of 2 amino acid polymorphisms adjacent and within the α2-β2 loop, an important structural motif modulating interspecies transmission of some prion strains [148], undermines their utility in testing the species barrier. In particular, the residue E168 in humans (Q168 in chimpanzees) appears to be fundamental for human reduced susceptibility to CWD and other prion strains from ruminants [149].

Transgenic mice expressing different variants of human PrP^C (MM129, MV129 and VV129) at 1–16-fold the levels expressed in the human brain were challenged with US and Canadian CWD isolates in seven different studies. Elegantly reviewed by Waddell et al., none of these studies found evidence of transmission to any of the transgenic mice (reviewed by [141]). Studies in chimeric mice suggested that the α2–β2 loop of the prion protein is the key to the transmission barrier of humans to CWD [148]. However, a recent study found low levels of RT-QuIC seeding activity in four mice overexpressing human prion protein (MM129) inoculated with elk and white-tailed deer isolates (2 mice per inoculation group). These results need to be interpreted with caution, as these reactions were inconsistently positive perhaps representing poorly adapted CWD prions into human PrP or alternatively, persistence of the inoculum in the brain of these mice since human prions can physically persist even in knock-out mice for extended periods post-inoculation [149]. In addition, these RT-QuIC positive mice (tg66) express the highest levels of human PrP tested in CWD transmission studies (8–16 × compared to human brain), which could facilitate the replication of CWD in human PrP [150]. In contrast, the same CWD isolates when inoculated into the tgRM (2–4×) resulted in less clinical suspects and no positive detection by RT-QuIC [150].

Finally, the transmission barrier of humans for CWD has been studied using ultra-sensitive in vitro techniques. The first in vitro study suggested a substantial molecular barrier limiting susceptibility of humans to CWD [151]. Davenport et al. demonstrated positive seeding activity when human recombinant PrP was seeded with CWD, but not when using BSE, contradicting results observed in vivo [152]. Successful conversion of human PrP using different CWD seeds in PMCA has been reported by Barria et al. Their studies suggest that CWD from MM132 elk and CWD from reindeer have the highest potential to convert human PrP, followed by white-tailed deer prions and, finally, mule deer CWD isolates, which require an intermediate step of in vitro conditioning to deer substrate [153–155]. This positive conversion was achieved, however, in PMCA reactions using a large CWD prions-to-human substrate ratio [154].

7 Conclusions

The prevalence and geographic spread of CWD continues to rise, expanding the likelihood of transmission to other species. Particularly of concern in North America is the risk to caribou, an endangered species. Although canids appear to have resistance to infection by CWD prions, other carnivores, i.e., the big felids, are predicted to be susceptible to infection. The zoonotic potential is still unclear but the increased prevalence of CWD in cervids will result in greater likelihood of human exposure.

Abbreviations

BSE: bovine spongiform encephalopathy; CJD: Creutzfeldt-Jakob disease; CNS: central nervous system; CWD: chronic wasting disease; DMNV: dorsal motor nucleus of the vagus nerve; dpe: days post-exposure; ENS: enteric nervous system; GALT: gut-associated lymphoid tissue; PMCA: protein misfolding cyclic amplification; PrP^{C}: cellular prion protein; PrP^{CWD}: cervid PrP^{Sc}; PrP^{Sc}: pathological prion protein; RT-QuIC: real-time quaking-induced conversion; TME: transmissible mink encephalopathy.

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Authors' contributions

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Declarations

Competing interests

The authors declare that they no competing interests.

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