RESEARCH ARTICLE

Inter- and intra-species conversion efficacies of Norwegian prion isolates estimated by serial protein misfolding cyclic amplification

Erez Harpaz[1†], Tram Thu Vuong[2†], Linh Tran[2], Michael Andreas Tranulis[3], Sylvie L. Benestad[2] and Cecilie Ersdal[1*]

Abstract

Prion diseases, including chronic wasting disease (CWD) in cervids, are fatal neurodegenerative disorders caused by the misfolding of cellular prion proteins. CWD is known to spread among captive and free-ranging deer in North America. In 2016, an outbreak of contagious CWD was detected among wild reindeer in Norway, marking the first occurrence of the disease in Europe. Additionally, new sporadic forms of CWD have been discovered in red deer in Norway and moose in Fennoscandia. We used serial protein misfolding cyclic amplification to study the ability of Norwegian prion isolates from reindeer, red deer, and moose (two isolates), as well as experimental classical scrapie from sheep, to convert a panel of 16 brain homogenates (substrates) from six different species with various prion protein genotypes. The reindeer CWD isolate successfully converted substrates from all species except goats. The red deer isolate failed to convert sheep and goat substrates but exhibited amplification in all cervid substrates. The two moose isolates demonstrated lower conversion efficacies. The wild type isolate propagated in all moose substrates and in the wild type red deer substrate, while the other isolate only converted two of the moose substrates. The experimental classical scrapie isolate was successfully propagated in substrates from all species tested. Thus, reindeer CWD and classical sheep scrapie isolates were similarly propagated in substrates from different species, suggesting the potential for spillover of these contagious diseases. Furthermore, the roe deer substrate supported conversion of three isolates suggesting that this species may be vulnerable to prion disease.

Keywords Cervids, CWD, Norway, PMCA, prion, *PRNP*, reindeer, scrapie, species-barrier, spillover

†Erez Harpaz and Tram Thu Vuong contributed equally to the work.

Handling editor: Vincent Béringue.

*Correspondence:
Cecilie Ersdal
cecilie.ersdal@nmbu.no
[1] Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Svebastadveien 112, 4325 Sandnes, Norway
[2] Department of Biohazard and Pathology, Norwegian Veterinary Institute, P.O. box 64, 1431 Ås, Norway
[3] Department of Preclinical Sciences and Pathology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Universitetstunet 3, 1433 Ås, Norway

Introduction

Chronic wasting disease (CWD) is a neurodegenerative disorder affecting several species of cervids [1]. The disease was first described in 1967 in a captive mule deer in Colorado, USA, and characterized later as a transmissible spongiform encephalopathy (TSE) [2]. The cause of these disorders is an alteration in the three-dimensional structure of the normal cellular prion protein (PrP^C), which is converted into a beta-sheet-rich, misfolded prion protein (PrP^{Sc}) and further amplified in a templated manner [3]. There are several known TSEs in both humans and animals, which, due to their shared etiology, are commonly referred to as prion diseases. In the contagious forms of

Harpaz *et al. Veterinary Research*   (2023) 54:84

Page 2 of 12

prion diseases, classical scrapie in small ruminants and classical CWD in cervids, disease associated prions can propagate in lymphoid tissues, and in later stages will disseminate in the central nervous system (CNS) where they cause spongiform changes [4, 5]. All prion diseases are fatal and end with the death of the host [6].

In 2016, the first reported classical CWD case in Europe was found in a free-ranging reindeer (*Rangifer tarandus tarandus*) in the Nordfjella reindeer area in the south of Norway [7]. This was also the first case of natural CWD infection in reindeer, although reindeer have been experimentally infected with CWD earlier [8, 9]. Following this event, due to intensifying monitoring, two additional cases of classical CWD were detected in reindeer in 2019 and 2022 in a previously unaffected region, the Hardangervidda plateau. In addition, sporadic CWD cases were found in moose (*Alces alces*) [10] and red deer (*Cervus elaphus elaphus*) [11] in several Norwegian counties. In total, 21 reindeer, 11 moose, and three red deer have to date been diagnosed with CWD in Norway [12], leaving roe deer (*Capreolus capreolus*) as the only native cervid species in Norway with no documented CWD cases. The CWD-positive reindeer cases consisted predominantly of young adults and adults with age range of 1–8 years. In 52.4% of the cases, PrP^{Sc} was found in both the lymph nodes and the CNS, while in the remaining of the animals, only the lymph node tested positive for PrP^{Sc} [12–14]. In contrast, Norwegian cases of moose and red deer with available age data exhibited a notably higher mean age of 14.9 years, with prions exclusively detectable in the CNS [10, 11, 13]. Besides the Norwegian cases, Sweden has reported four cases of moose CWD, while Finland has reported three cases [15, 16]. Detailed analysis revealed significant differences in disease characteristics between moose and reindeer cases, as well as between moose cases, all distinct from the well-established North American CWD isolates [10, 15, 17], including their potential for interspecies transmission [17, 18].

The "species barrier" refers to the inefficiency of a prion disease to transmit and establish in a different species [19, 20]. Phylogenetically related species may transmit prion diseases more easily such as scrapie between goats and sheep and CWD between different cervids [21]. However, prion diseases can sometimes be transmitted to distantly related species and may also have zoonotic potential, as exemplified by the tragic emergence of variant Creutzfeldt-Jakob disease (vCJD) in humans, which derived from bovine spongiform encephalopathy (BSE) and overcame the species barrier [22, 23]. Susceptibility to most prion diseases is influenced by prion protein gene (*PRNP*) polymorphisms which, in contagious diseases, can determine the fate of the prion within the host following uptake. Similarly,

PRNP variation will influence the likelihood of spontaneous conversion of PrP^C in the brain [24–27].

The presence of multiple prion strains, even within a single host, may account for the manifestation of varied disease phenotypes, despite the host's PrP^C having a similar primary structure [28]. According to the conformation selection theory, a "strain" is, in fact, a group of prions with diverse three-dimensional shapes and biomolecular characteristics. Some of these conformers may be compatible with the target species PrP^C, which may explain how certain strains, or isolates, can cross the species barrier and cause disease. On the other hand, if none of the prion conformers within the host are compatible with the target species PrP^C, transmission will not occur [29].

Norway has abundant wild and semi-domesticated reindeer as well as free-roaming moose, roe deer, and red deer. These cervids exhibit migratory behavior [30], making their movements and interactions with other species difficult to control. During summers, sheep are released to graze in mountain pastures, and as demonstrated in Nordfjella, sheep and reindeer spatial overlapping exists, hence the potential risk of CWD spillover to other species [31, 32].

Several species have been experimentally inoculated with different North American CWD isolates [33–37], but inoculation studies using Norwegian CWD isolates are sparse, and so far, only experiments in bank vole and genetically modified mice have been published [17, 38, 39].

Protein misfolding cyclic amplification (PMCA) can be employed to offer in-vitro data on prion replication when inoculation studies are missing. This technique involves repeated cycles of incubation and sonication, where a PrP^{Sc} seed, or isolate, is added to a PrP^C-rich substrate (usually homogenized normal brain) to start prion conversion. In the sonication phase, prion aggregates are thought to be fragmented, exposing more reactive sites, and increasing the seeding conversion activity in the subsequent incubation phase. These incubation-sonication cycles continue until the PrP^C content in the substrate is exhausted, usually after 48 h [40]. An aliquot of the product can then be added to a fresh substrate which in turn can be subjected to a new round of cyclic sonication and incubation called serial, or sPMCA.

The objective of this study was to assess the conversion potential of various Norwegian CWD isolates towards several cervid species as well as domestic small ruminants. We utilized prion isolates from CWD-affected reindeer, moose, and red deer and an experimental classical sheep scrapie isolate, as seeds in sPMCA reactions. As substrates, we used brain homogenates from the four

Harpaz *et al. Veterinary Research*   (2023) 54:84

Page 3 of 12

cervid species native to Norway, as well as sheep and goats with different *PRNP* genotypes.

Materials and methods
Source of brain substrates
Reindeer brains, except for the one with the wild type *PRNP*, were collected during the slaughter of semi-domesticated reindeer from Nordland County in northern Norway. The wild type brain was collected during the slaughter of semi-domesticated reindeer from the Filefjell reindeer herding unit. The brains of the other cervid species were acquired during the autumn 2020 hunting season. The cervid brains were frozen at −20 °C as soon as possible after collection and later transferred to a −70 °C freezer. Fresh, healthy sheep brains were collected during necropsies performed at the Section for Small Ruminant Research and Herd Health, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, or immediately after slaughter at the Nortura Forus abattoir. These brains were frozen at −70 °C immediately after sampling. The goat brains were obtained from a previous experiment [41]. All brain substrates in the study were tested negative for PrP^{Sc} by Enzyme-Linked Immunosorbent Assay (HerdCheck BSE/scrapie Ag test, IDEXX). The brain substrates used in the experiment are summarized in Table 1.

PRNP genotyping
Deoxyribonucleic acid (DNA) was extracted from either blood or brain tissue using the DNeasy Blood & Tissue Kit (Qiagen GmbH) following the manufacturer's instructions. Cervid *PRNP* genotypes were determined by sequencing the plus strand of the *PRNP* open reading frame after PCR amplification using the following primers: forward 5′-ATTTTGCAGATAAGTCAT CATG-3′ and reverse 5′-AGAAGATAATGAAAACAG GAAG-3′. The PCR reaction included 5 μL of genomic DNA as a template, 25 μL of DreamTaq Green PCR Master Mix (Thermo Fisher Scientific), 2.5 μL of forward and reverse primers (10 μM) (Thermo Fisher Scientific), and nuclease-free water to reach a final volume of 50 μL. The PCR amplification consisted of an initial cycle at 95 °C for 3 min, followed by 36 cycles at 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 7 min. The coding region of the goat *PRNP* was analyzed similarly using the following primers: forward 5′-GAT GCCACTGCTATGCAGTCAT-3′ and reverse 5′-AAA ACAGGAAGGTTGCCCCT-3′. For sheep, selective *PRNP* genotyping at amino acid positions 136, 154, and 171 was performed by a commercial laboratory (Eurofins Genomic GmbH). The accession numbers for the wild type *PRNP's* are ACT87519.1 for sheep, MN784961.1 for reindeer, JQ290077.1 for moose, MK103027.1 for red deer, and MK103016.1 for roe deer.

Prion isolates
The isolates used as seeds are listed in Table 2. The reindeer CWD isolate originates from the Nordfjella outbreak and has not been characterized earlier. The moose and red deer isolates were identified through the national surveillance program for CWD. The wild type moose isolate, ID CD11399 [10, 17, 18, 39] and red deer isolate ID CD14051 [18] have been previously characterized. The sheep scrapie isolate originates from an experimental study [42].

Serial protein misfolding cyclic amplification procedure
To prepare the substrates, brain tissues were homogenized using a Potter–Elvehjem tissue grinder with a conversion buffer consisting of 1X PBS, 150 mM NaCl, 1%

Table 1 Brains used as sPMCA substrates and their respective *PRNP* genotypes.

Species	*PRNP* genotype
Reindeer	S_{225}/S_{225} (wild type)
	Y_{225}/Y_{225}
	D_{176}/D_{176}
	$M_2S_{129}M_{169}/M_2S_{129}M_{169}$
	S_{225}/Y_{225}
Red deer	Q_{226}/Q_{226} (wild type)
	E_{226}/E_{226}
Roe deer	Q_{226}/Q_{226} (wild type)
Moose	K_{109}/K_{109} (wild type)
	Q_{109}/Q_{109}
	Q_{109}/K_{109}
Sheep	$A_{136}R_{154}Q_{171}/A_{136}R_{154}Q_{171}$ (wild type)
	$V_{136}R_{154}Q_{171}/V_{136}R_{154}Q_{171}$
	$A_{136}R_{154}R_{171}/A_{136}R_{154}R_{171}$
Goat	Q_{220}/L_{220}
	Ter_{32}/Ter_{32} (no PrP^C expression)

Table 2 Prion isolates used as seed in the sPMCA experiments.

Species	ID	*PRNP* genotype	Type of isolate	Tissue
Reindeer	CD24089	S_{225}/S_{225} (wild type)	Contagious	Lymph node
Moose	CD11399	K_{109}/K_{109} (wild type)	Sporadic	Brain
Moose	CD24854	Q_{109}/Q_{109}	Sporadic	Brain
Red deer	CD14051	E_{226}/E_{226}	Sporadic	Brain
Sheep	7233	$V_{136}R_{154}Q_{171}/V_{136}R_{154}Q_{171}$	Contagious	Brain

Harpaz *et al. Veterinary Research*   (2023) 54:84

Page 4 of 12

Triton X-100, and a protease inhibitor cocktail (Roche) in a 1/10 ratio. The homogenates were then centrifuged at 800 g at 4 °C for 2 min. After centrifugation, the supernatant was transferred to 1 mL Eppendorf tubes and frozen at −70 °C. Before starting the PMCA procedure, the substrate tubes were thawed, and 100 µg/mL heparin and 0.05% digitonin were added. A mixture of 10 µL of seed and 90 µL of substrate was added to a PCR tube containing two Teflon beads and then placed in a sonicator horn connected to a Q700 sonicator (Qsonica). The mixture underwent 48 h of sonication-incubation cycles, which involved 30 s of sonication at 230-250W and 29 min and 30 s of incubation at 37 °C. After each 48-h cycle, the procedure was repeated using 10 µL of the product added to 90 µL of fresh substrate. Each 48-h sonication-incubation cycle was considered one round of PMCA. For the PMCA experiments, we included negative controls consisting of normal brain homogenate (substrate) seeded with PrP^{Sc}-negative brain or lymph node (reindeer) homogenate. The source of the PrP^{Sc} negative seeds corresponded to the species from which the PrP^{Sc}-positive seed used in that experiment originated. In addition, at least one tube with non-seeded brain homogenate was included in each experiment to monitor for potential cross-contamination.

Proteinase K digestion and western blotting

Western blot was performed on the original isolates, some of the substrates, and the PMCA products using the TeSeE Western Blot kit (Bio-Rad) following the manufacturer's instructions. The tested PMCA products and the isolates were subjected to a one-hour digestion with 100 µg/mL proteinase K (PK) (Sigma-Aldrich) at 37 °C. This step was omitted for the cervid substrates to detect the host PrP^C. The reaction was stopped by incubating the mixture with Laemmli sample buffer (Bio-Rad) for five minutes at 100 °C. The samples were then loaded onto Sodium Dodecyl Sulfate–Polyacrylamide gel (NuPAGE 12% Bis–Tris protein gels, Thermo-Fisher) and electrophoresed at 100 V for 55 min. The proteins were subsequently transferred to a polyvinylidene difluoride membrane using a trans-blot turbo system (Bio-Rad), and the membrane was then subjected for immunodetection. The commercial kit included the primary monoclonal antibody (mAb), Sha31, which recognizes the core epitope 148YEDRYYRE155. Additionally, the mAb P4 (Labolytic), which identifies the N-terminal epitope 93WGQGGSH99 (ovine numbering for both antibodies), was used. The results were visualized using Azure c280 (Azure Biosystems). Brains from a scrapie-positive sheep, either alone or together with a moose CWD isolate, were utilized as positive controls.

Results

sPMCA using normal brain homogenate as substrate

We tested the ability of prion isolates from Norwegian cervids and sheep to be amplified in brain homogenates from different species with different *PRNP* genotypes. We used a total of five different isolates as seeds, derived from four species: reindeer, moose (two isolates), red deer, and sheep (Table 2). As substrates, we utilized brain homogenate from six different species with different *PRNP* genotypes, totaling 16 brain homogenates (Table 1), some of which represented the wild type *PRNP* for the respective species. For each species, and when applicable, we employed substrates with varying susceptibility to prion disease based on current knowledge. We also performed western blotting on the cervid brain homogenates to confirm that P4 and Sha31 mAbs are suitable for detecting cervid PrP^C. These antibodies target two different regions on the prion protein: Sha31 targets the core region, and P4 targets the N-terminal region. Both antibodies recognized PrP^C in all tested species (Figure 1A). We observed that the contagious isolates from reindeer CWD and experimental sheep scrapie exhibited strong conversion abilities (Figures 2 and 3, Table 3). Interestingly, the reindeer isolate only converted the wild type reindeer substrate and none of the other reindeer substrates. The red deer isolate also converted substrates from all cervids, but not the sheep and goat brains. The moose wild type isolate converted all moose substrates as well as the red deer wild type brain, while the moose Q_{109}/Q_{109} isolate only converted two of the moose substrates. The roe deer substrate was converted by three out of five seeds, including both contagious CWD isolates. As expected, no conversion was observed in the scrapie resistant $A_{136}R_{154}R_{171}/A_{136}R_{154}R_{171}$ sheep substrate and the goat Ter_{32}/Ter_{32} substrate without expression of cellular PrP (Figure 3). The complete results are presented in Table 3.

Biochemical property characterization of sPMCA products

Since the moose and red deer CWD isolates have different N-terminal PK-cleavage sites compared to the contagious prion isolates [10, 16], we wanted to further characterize the included isolates. The Sha31 antibody detects all PrP^{Sc} present, while the P4 antibody only recognizes products which retain this epitope of the N-terminal following PK treatment. Following PK digestion of the non-amplified isolates, both the wild type moose and the red deer isolates lost their P4 epitope, while the other isolates maintained it (Figure 1B).

We observed that the reindeer isolate lost the P4 epitope following amplification in the Q_{109}/Q_{109} and the K_{109}/Q_{109} moose substrates (Figure 4A), while the other contagious seed, the classical scrapie, kept its P4 epitope in all amplified substrates (Figures 4A–D). The
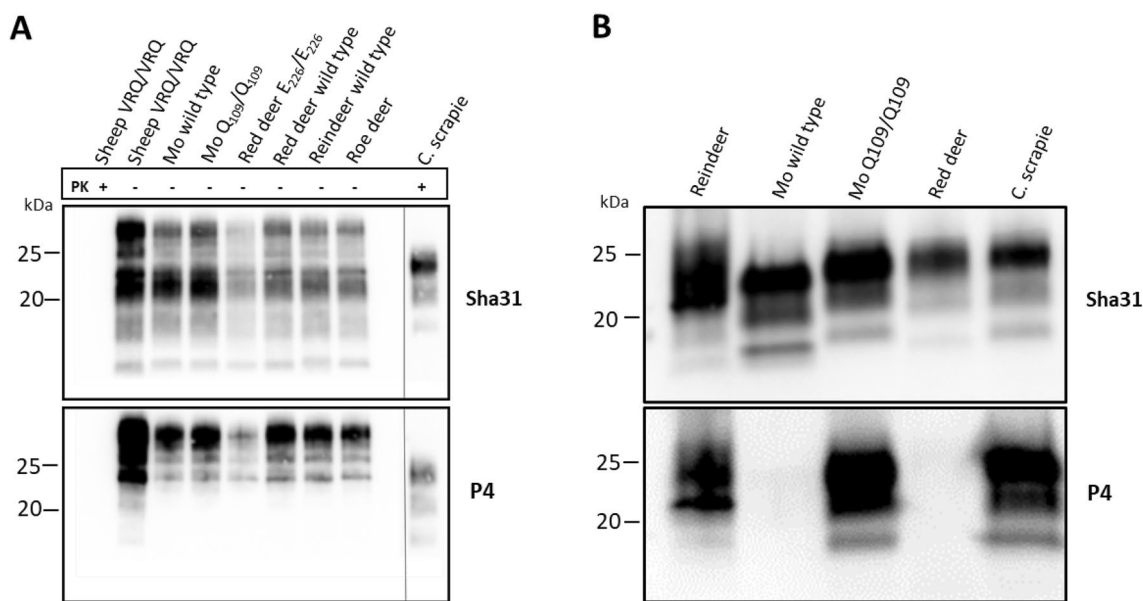
Harpaz *et al. Veterinary Research*   (2023) 54:84

Page 5 of 12

Figure 1 **Western blot analysis of cervid substrates and prion isolates for PrP content with monoclonal antibodies Sha31 and P4**.
A Both Sha31 and P4 effectively identified PrP^C in brain homogenates of all cervid species in the study. Three control samples were included: normal VRQ/VRQ sheep homogenate with and without proteinase K (PK) treatment and a VRQ/VRQ classical scrapie with prion banding pattern after PK treatment. **B** The same antibodies were used to visualize the original prion isolates included in the study. The P4 mAb did not detect the wild type moose and red deer isolates. The two contagious isolates, but also the sporadic moose Q_{109}/Q_{109} isolate kept the P4 epitope. All samples were subjected to PK treatment. *VRQ* $V_{136}R_{154}Q_{171}$, *Mo* moose, *C. scrapie* classical scrapie, *kDa* molecular weight in kilodalton.

signal intensity of the amplified moose wild type isolate in moose wild type substrate was strongly reduced with P4 mAb, and the P4 epitope was completely lost in the two other moose substrates. In contrast, the moose Q_{109}/Q_{109} isolate kept its P4 epitope in the Q_{109}/Q_{109} and the K_{109}/Q_{109} moose substrates (Figure 4A). All PMCA products of the red deer isolate lost the P4 epitope (Figures 4A–D).

Discussion

In this study, we estimated conversion efficacies of five distinct Norwegian prion isolates using sPMCA. Two of the included isolates cause contagious diseases: one classical CWD isolate from reindeer and one from sheep with experimental classical scrapie. In addition, three isolates of sporadic prion diseases were included: one from red deer and two isolates from moose [16]. We found that prions from all isolates could be amplified in at least some of the brain substrates, and the isolates of the contagious diseases were the most efficient. The conversion efficacy was not only dependent on the origin of the isolate but also on the *PRNP* variant of the tested substrate.

Following the CWD outbreak in 2016, the entire reindeer herd in Nordfjella was culled, leaving the area reindeer-free [43], while the Hardangervidda plateau, the second affected area, still maintains a closely regulated reindeer population [44]. Both these areas are used as summer grazing pastures for sheep. Since concern exists

for prion spillover, 500 sheep that had grazed in Nordfjella were investigated, and no prions were found in their gut-associated lymphoid tissue, despite habitat overlapping with diseased reindeer [31]. In experimental settings, classical North American CWD isolates overcame the species barrier when inoculated intracerebrally into scrapie susceptible sheep, albeit with a low transmission rate [37]. However, at the second passage, there was a 100% attack rate [45]. Inoculation by the oronasal route with a CWD isolate from white-tailed deer (WTD), resulted in transmission to only one of seven sheep carrying the scrapie susceptible $A_{136}R_{154}Q_{171}$/$A_{136}R_{154}Q_{171}$ genotype (wild type) after a long incubation time [46]. Based on these studies, sheep may have a low risk contracting North American CWD, but it is important to note that characterization of the Norwegian isolates so far indicate that these are different from the North American isolates [17, 18, 39].

One PMCA study that used substrates from transgenic ovinized mice carrying the $A_{136}R_{154}Q_{171}$/$A_{136}R_{154}Q_{171}$ genotype indicated that reindeer CWD has a relatively strong spillover potential towards sheep, but also the sporadic red deer isolate showed the same tendency, while the Norwegian moose CWD strain did not [18]. Interestingly, both the wild type moose isolate, and the red deer isolate characterized in that paper are the same as those utilized in the present study, but none of
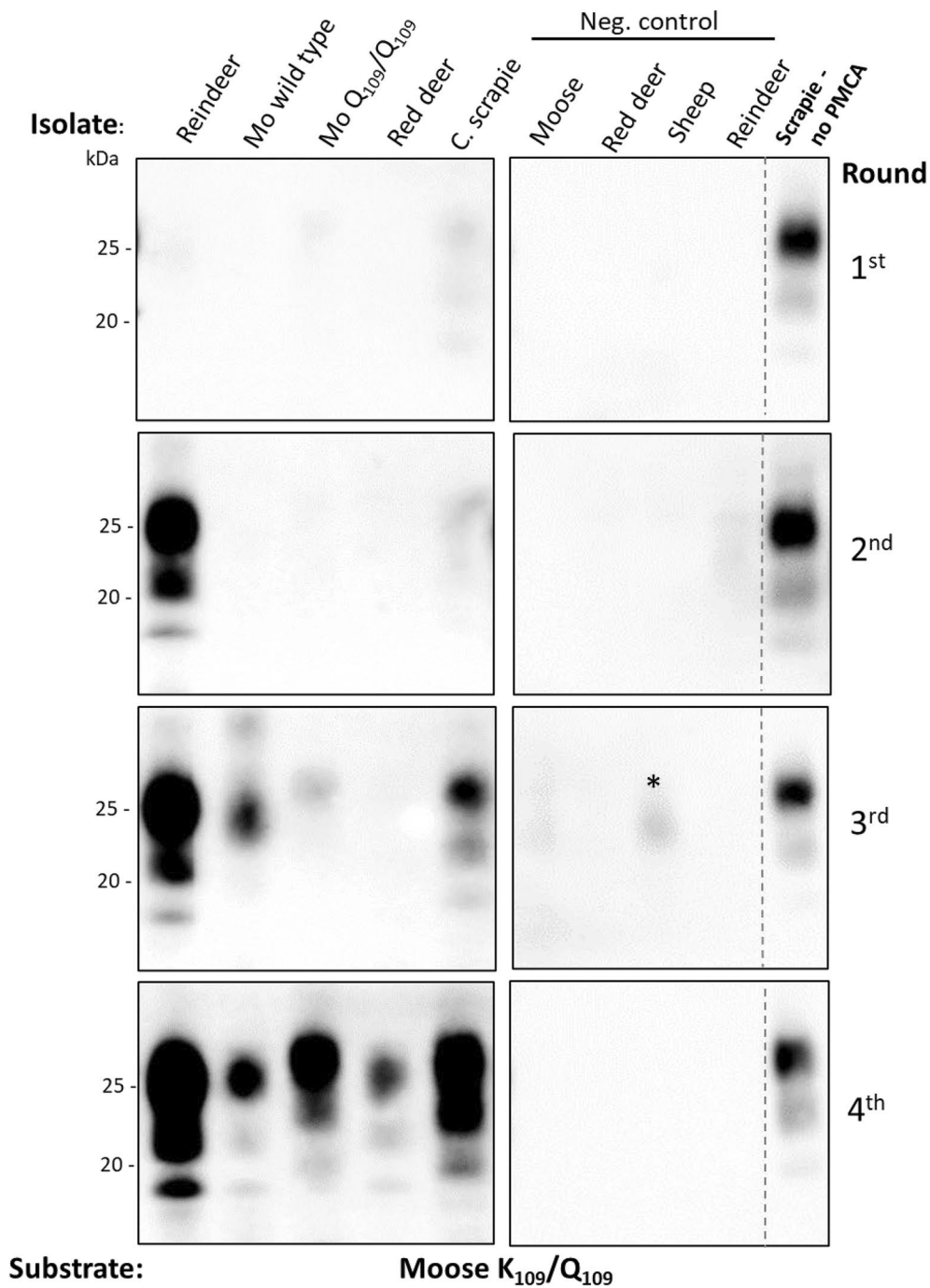
Harpaz *et al. Veterinary Research*   (2023) 54:84

Page 6 of 12

Figure 2 Representative western blots showing sPMCA of Norwegian CWD and experimental classical scrapie isolates in the moose brain substrate K_{109}/Q_{109} variant. Cervid CWD and experimental classical scrapie isolates were subjected to sPMCA using moose K_{109}/Q_{109} brain as substrate. After each PMCA round, the amplified products were digested with proteinase K and analyzed by western blotting. After four rounds of PMCA, all the isolates were amplified and characteristic prion bands could be seen in the western blots. *Mo* moose, *C. scrapie* experimental classical scrapie, * Unspecific band, *kDa* molecular weight in kilodalton.

these isolates amplified in our three sheep substrates. We found weak conversion of the reindeer isolate in both scrapie susceptible $A_{136}R_{154}Q_{171}/A_{136}R_{154}Q_{171}$ and

$V_{136}R_{154}Q_{171}/V_{136}R_{154}Q_{171}$ substrates, comparable to the amplification of a different Norwegian CWD affected reindeer in ovinized mice brain [18]. This could indicate

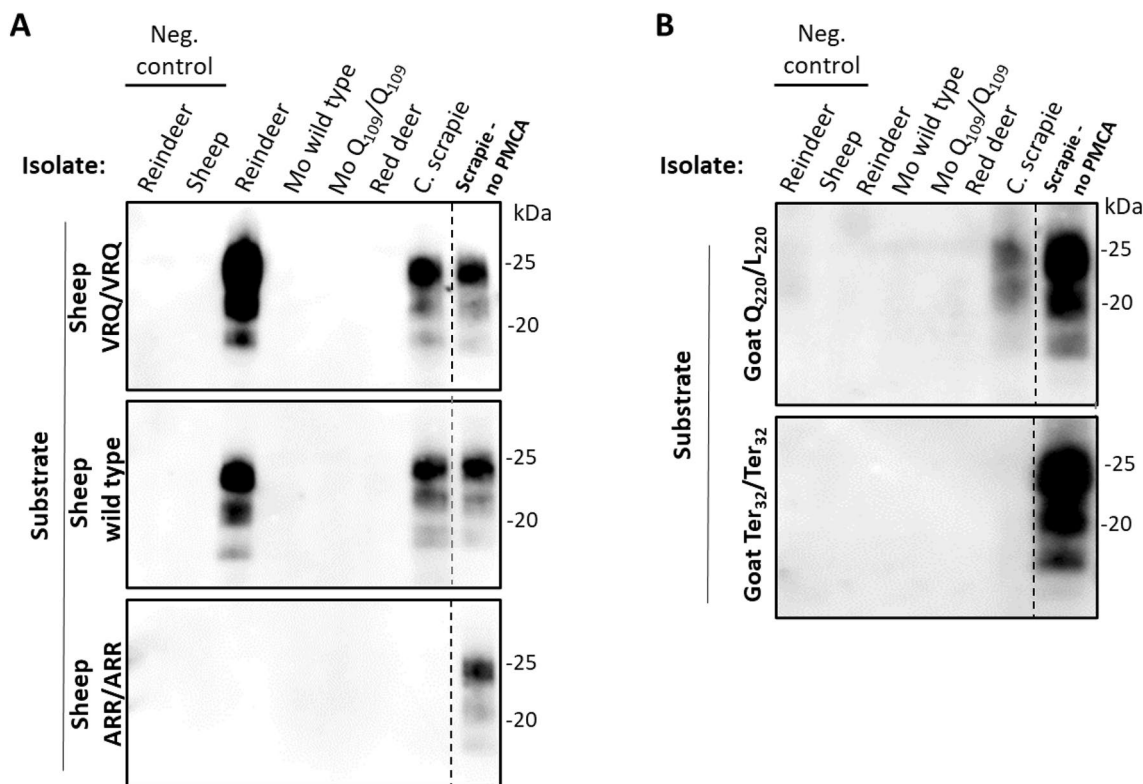Harpaz *et al. Veterinary Research*   (2023) 54:84

Page 7 of 12

Figure 3 Amplification of Norwegian CWD and experimental classical scrapie isolates in sheep and goat substrates after 8 rounds of PMCA. A Only the reindeer and scrapie isolates were amplified in VRQ/VRQ and ARQ/ARQ sheep substrates. **B** Only the experimental classical scrapie isolate was amplified in goat Q_{220}/L_{220} substrate. None of the isolates were amplified in the sheep brain with the most scrapie resistant genotype ARR/ARR and the goat Ter_{32}/Ter_{32} substrate without expression of the cellular prion protein. *VRQ/VRQ* $V_{136}R_{154}Q_{171}/V_{136}R_{154}Q_{171}$, *ARR/ARR* $A_{136}R_{154}R_{171}/A_{136}R_{154}R_{171}$, *Mo* moose, *C. scrapie* experimental classical scrapie, *kDa* molecular weight in kilodalton.

a possibility for reindeer CWD spillover to sheep and in line with the North American studies (37, 46). Unexpectedly, the scrapie isolate was only weakly amplified in the goat substrate, which also appeared resistant to the cervid isolates. Several goat *PRNP* polymorphisms have been reported, and some genotypes have been associated with reduced susceptibility to classical scrapie. In this study, we utilized goat substrate with the Q_{220}/L_{220} genotype, which does not appear to alter susceptibility to classical scrapie [47]. As expected, regardless of the isolate used, no conversion was observed in the scrapie resistant $A_{136}R_{154}R_{171}/A_{136}R_{154}R_{171}$ sheep substrate and the prion protein deprived Ter_{32}/Ter_{32} goat substrate.

The newly emerging Norwegian reindeer CWD isolate presents a probable risk to other cervids, while the sporadic isolates are assumed to have a low risk of transmission since they are probably not contagious. Norway has substantial populations of wild cervids, encompassing four native species [30]. These wild cervids exhibit a well described spatial migration [48–50] and can potentially encounter environmental prions excreted by diseased reindeer. The reindeer isolate converted all four cervid species, and the experimental scrapie isolate had comparable amplification efficacy indicating that these two contagious diseases have a spillover potential to these species.

There are at least three different moose CWD isolates [15, 16, 39] in Fennoscandia and the disease appears so far in animals with either the wild type *PRNP*)K_{109}/K_{109}) or the Q_{109}/Q_{109} variant. The two tested moose isolates in the present study were able to amplify all but one of the moose substrates. The wild type isolate also converted the wild type red deer substrate with identical *PRNP* sequence. When the same wild type isolate was tested against brain homogenates from transgenic mice expressing the deer (wild-type) or elk prion protein, both were amplified [18]. In yet another study, this isolate showed efficient transmission to Tg-mice expressing the cervid wild type *PRNP*, but slower and incomplete to Tg-E_{226} mice [39].

The European red deer population in Norway possesses only one *PRNP* polymorphism at position 226, in contrast to red deer in other European countries where multiple variants exist [51]. In North America, where European

Harpaz *et al. Veterinary Research*   (2023) 54:84

Page 8 of 12

Table 3 sPMCA conversion results.

Brain substrate		Prion isolate				
Species	*PRNP*-genotype	sPMCA amplification				
		Reindeer CWD	Moose CWD		Red deer CWD	Sheep scrapie
		wt	wt	Q_{109}/Q_{109}	E_{226}/E_{226}	V_{136}/V_{136}
Reindeer	S_{225}/S_{225} (wt)	+ + + +	−	−	+	+ + +
	S_{225}/Y_{225}	−	−	−	−	−
	Y_{225}/Y_{225}	−	−	NA	NA	NA
	D_{176}/D_{176}	−	−	NA	NA	NA
	$M_2S_{129}M_{169}/M_2S_{129}M_{169}$	−	−	NA	NA	NA
Moose	K_{109}/K_{109} (wt)	+ + +	+ +	-	+ + +	+ + +
	Q_{109}/Q_{109}	+ + + +	+ + +	+ +	+ + +	+ + + +
	K_{109}/Q_{109}	+ + + +	+ + +	+ + +	+ + +	+ + +
Red deer	Q_{226}/Q_{226} (wt)	+ + +	+ +	−	+ +	+ + + +
	E_{226}/E_{226}	+ + + +	−	−	+ +	+ + + +
Roe deer	Q_{226}/Q_{226} (wt)	+ +	−	−	+ +	+ + +
Sheep	$A_{136}R_{154}Q_{171}/A_{136}R_{154}Q_{171}$ (wt)	+	−	−	−	+ + + +
	$V_{136}R_{154}Q_{171}/V_{136}R_{154}Q_{171}$	+	−	−	−	+ + +
	$A_{136}R_{154}R_{171}/A_{136}R_{154}R_{171}$	−	−	−	−	−
Goat	Q_{220}/L_{220}	−	−	−	−	+
	Ter_{32}/Ter_{32}	−	−	−	−	−

The grading indicates the PMCA round of first detectable amplification by western blot, following digestion with proteinase K (100 µg/mL final concentration): +, round 7–8; + +, rounds 5–6; + + +, round 3–4; + + + +, round 1–2;—– no amplification; *NA* not performed, *wt* wild type, V_{136}/V_{136}—$V_{136} R_{154} Q_{171}/V_{136} R_{154} Q_{171}$, Ter_{32}/Ter_{32} no expression of PrP^C.

red deer have been introduced for recreational purposes, only a few cases of contagious CWD have been reported [52, 53]. Apart from these infrequent naturally occurring cases, four red deer with three different genotypes at position 226 were orally inoculated with a CWD isolate from Rocky Mountain elk. All animals developed clinical disease with detectable prions in their brains and lymphoid tissue at similar time point after inoculation, irrespective of the *PRNP* genotype [54]. The three sporadic CWD cases from Norway occurred in animals carrying the E_{226}/E_{226} genotype [11, personal communication Sylvie L. Benestad]. Notably, the red deer isolate amplified in all cervid species, and with high efficacy in the moose substrate and the homologous red deer substrate. This contrasts with the other two sporadic isolates included in the study. In line with our results, the same red deer isolate had efficient amplification in two transgenic mice substrates with the wild type and E_{226}/E_{226} variant *PRNP* [18]. Some of the results concerning the sporadic isolates may seem surprising and show that the behavior of these isolates under PMCA conditions may be less predictable than that of the contagious isolates.

Roe deer is the only species in our experiment with no reports of naturally occurring CWD, and to our knowledge, no inoculation studies have been performed in this species. Roe deer seems to maintain *PRNP* homology

overall [51, 55, 56], and only one conference paper has reported a polymorphism in three roe deer in Portugal [57]. Interestingly, our study showed that three of the prion isolates amplified in the roe deer substrate, particularly the reindeer and scrapie isolates showing good conversion efficacy. These results suggest that attention should be given to this species concerning surveillance for CWD. In Norway, the roe deer population is quite large, estimated to be around 115 000 animals [30]. However, only a few animals were reported hunted in the municipalities around Nordfjella, and the spatial overlap of roe deer and reindeer is thought to be low [32]. While the roe deer population in Norway is part of the national surveillance program for CWD, it is noteworthy that the annual testing rate for these free-ranging cervids is the lowest compared to other species [58].

Norway is the only country in Western Europe with wild reindeer populations. Additionally, large populations of semi-domesticated reindeer can be found in the middle and north of the country. The reindeer exhibit a particularly diverse *PRNP* variation. The subspecies found in the Fennoscandian Peninsula, the Eurasian reindeer (*Rangifer tarandus tarandus*), was discovered to have seven non-synonymous base pair substitutions and one octapeptide deletion [56]. To date, 17 different genotypes have been identified among wild and semi domesticated
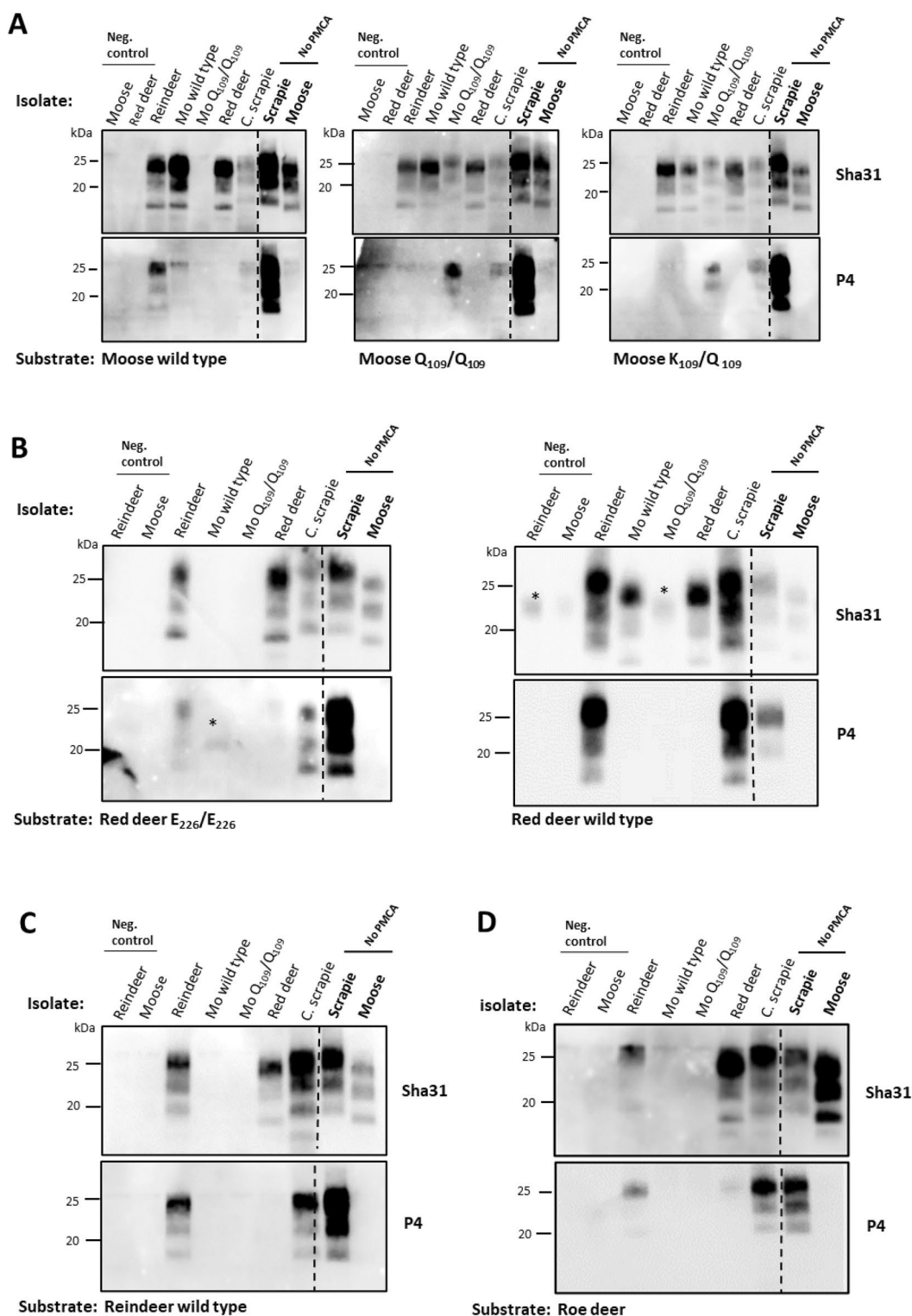
Harpaz *et al. Veterinary Research* (2023) 54:84

Page 9 of 12

Figure 4 Biochemical properties of the PMCA products by discriminating primary antibodies. The Sha31 antibody, recognizing the core region and the P4 antibody targeting the N-terminal of the PrP^{Sc} were used to assess the biochemical properties of the amplified products in **A** moose, **B** red deer, **C** reindeer and **(D)** roe deer substrates. The amplified PrP^{Sc} exhibited properties similar to those of the original isolates except for the reindeer isolate that lost the P4 epitope in the moose Q_{109}/Q_{109} and K_{109}/Q_{109} substrates. * Unspecific band; *Mo* moose. *C. scrapie* experimental classical scrapie, *kDa* molecular weight in kilodalton.

Harpaz *et al. Veterinary Research*   (2023) 54:84

Page 10 of 12

reindeer in Norway. Contagious CWD in Norwegian reindeer has only been found in the wild type *PRNP* and in three different heterozygote variants. The wild type and the heterozygote wild type/octapeptide deletion variants seem to be equally susceptible to the disease [59]. The wild type *PRNP* is more common among wild reindeer than the semi-domesticated reindeer in Norway. Additionally, the octapeptide deletion is very rare among the semi-domesticated herds. Instead, these animals carry higher frequencies of genotypes which seem to be less susceptible to CWD [56]. The Eurasian reindeer has been successfully infected with North American CWD via the oral route [8, 9]. In one of the studies, reindeer were inoculated with CWD isolates from either elk or WTD, and only those that were inoculated with WTD CWD did accumulate prions in their brains and in peripheral organs. The *PRNP* of the inoculated animals was a variant not found in the Norwegian reindeer populations [8]. In our study, we discovered that both reindeer and experimental scrapie isolates induced conversion in the reindeer wild type substrate. Unexpectedly, the S_{225}/Y_{225} substrate was not converted by any of the five isolates, despite several reported cases of natural CWD infection in this variant [59]. Furthermore, we observed no conversion activity in the Y_{225}/Y_{225}, D_{176}/D_{176}, and $M_2S_{129}M_{169}/M_2S_{129}M_{169}$ reindeer substrates, which are genotypes not reported among the positive natural cases, when tested against the reindeer and moose wild type isolates, the only two isolates examined.

Finally, we used two monoclonal antibodies to further characterize the PMCA products and the PK cleavage sites of the isolates. We demonstrated loss or strong signal reduction of the N-terminal segment including the P4 epitope in the Norwegian moose CWD isolates across most substrates in accordance with an earlier study [10]. We noted that the amplified product of the previously uncharacterized Q_{109}/Q_{109} isolate maintained the P4 epitope, as seen in the original isolate, in two of the moose substrates. Intriguingly, the rein deer isolate lost the P4 epitope when amplified in the same two moose substrates, while the scrapie and red deer retained their original signatures across all substrates. These findings indicate that, although, in most cases the cleavage site of PMCA products seems to be determined by the isolate itself, a molecular shift can be induced in the substrate that could possibly be explained by reduced compatibility between the seed and the substrate or some unknown characteristics of the isolate [60].

In these sPMCA experiments, we observed that prion isolates obtained from Norwegian reindeer can convert a range of substrates from different species with an efficacy comparable to that of an experimental classical scrapie isolate, suggesting a potential for spillover of these contagious diseases. Conversely, the conversion efficacy of isolates derived from moose and red deer, representing sporadic forms of CWD, was weaker than that of the contagious isolates. Still, three of the cervid isolates converted substrates of other cervid species suggesting a weaker species barrier and a transmission potential between these species. We also demonstrated that roe deer substrate was converted by three different CWD isolates, indicating that this species may be susceptible to prion disease and warrants closer attention.

Authors' contributions
CE, SLB, MT, EH and TTV planned the experiment. EH and CE were responsible for obtaining the substrates. TTV performed the main laboratory work. EH and LT assisted with laboratory work. EH drafted the manuscript. CE participated in the interpretation of data and drafting of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

References
1. Escobar LE, Pritzkow S, Winter SN, Grear DA, Kirchgessner MS, Dominguez-Villegas E, Machado G, Townsend Peterson A, Soto C (2020) The ecology of chronic wasting disease in wildlife. Biol Rev Camb Philos Soc 95:393–408
2. Williams ES, Young S (1980) Chronic wasting disease of captive mule deer: a spongiform encephalopathy. J Wildl Dis 16:89–98
3. Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. Science 216:136–144
4. Jeffrey M, González L (2004) Pathology and pathogenesis of bovine spongiform encephalopathy and scrapie. Curr Top Microbiol Immunol 284:65–97
5. Williams ES (2005) Chronic wasting disease. Vet Pathol 42:530–549
6. Sigurdson CJ, Bartz JC, Glatzel M (2019) Cellular and molecular mechanisms of prion disease. Annu Rev Pathol Mech Dis 14:497–516
7. Benestad SL, Mitchell G, Simmons M, Ytrehus B, Vikøren T (2016) First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer. Vet Res 47:88
8. Mitchell GB, Sigurdson CJ, O'Rourke KI, Algire J, Harrington NP, Walther I, Spraker TR, Balachandran A (2012) Experimental oral transmission of

chronic wasting disease to reindeer (*Rangifer tarandus tarandus*). PLoS One 7:e39055

9. Moore SJ, Kunkle R, Greenlee MH, Nicholson E, Richt J, Hamir A, Waters WR, Greenlee J (2016) Horizontal transmission of chronic wasting disease in reindeer. Emerg Infect Dis 22:2142–2145

10. Pirisinu L, Tran L, Chiappini B, Vanni I, Di Bari MA, Vaccari G, Vikøren T, Madslien KI, Våge J, Spraker T, Mitchell G, Balachandran A, Baron T, Casalone C, Rolandsen CM, Røed KH, Agrimi U, Nonno R, Benestad SL (2018) Novel Type of chronic wasting disease detected in moose (*Alces alces*), Norway. Emerg Infect Dis 24:2210–2218

11. Vikøren T, Våge J, Madslien KI, Røed KH, Rolandsen CM, Tran L, Hopp P, Veiberg V, Heum M, Moldal T, Neves CGD, Handeland K, Ytrehus B, Kolbjørnsen Ø, Wisløff H, Terland R, Saure B, Dessen KM, Svendsen SG, Nordvik BS, Benestad SL (2019) First detection of chronic wasting disease in a wild red deer (*Cervus elaphus*) in Europe. J Wildl Dis 55:970–972

12. Skrantesjukestatistikk (in Norwegian) http://apps.vetinst.no/skrantesyk estatistikk/NO/ Accessed 25 June 2023

13. Ytrehus B, Asmyhr M, Hansen H, Mysterud A, Nilsen EB, Strand O, Tranulis MA, Våge J, Kapperud G, Madslien K, Rueness EK, Wasteson Y (2021) Mitigation options after detection of chronic wasting disease in the Hardangervidda wild reindeer area—a basis for future management strategies. Scientific opinion from the Norwegian scientific committee for food and environment (VKM). Oslo, Norway https://vkm.no/download/ 18.70b8d03018379c5cdba2398e/1664343084246/CWD%202020_engli sh.pdf Accessed 28 June 2023

14. Mysterud A, Madslien K, Viljugrein H, Vikøren T, Andersen R, Güere ME, Benestad SL, Hopp P, Strand O, Ytrehus B, Røed KH, Rolandsen CM, Våge J (2019) The demographic pattern of infection with chronic wasting disease in reindeer at an early epidemic stage. Ecosphere 10:e02931

15. Sun JL, Kim S, Crowell J, Webster BK, Raisley EK, Lowe DC, Bian J, Korpenfelt SL, Benestad SL, Telling GC (2023) Novel prion strain as cause of chronic wasting disease in a moose, Finland. Emerg Infect Dis 29:323–332

16. Tranulis MA, Gavier-Widén D, Våge J, Nöremark M, Korpenfelt SL, Hautaniemi M, Pirisinu L, Nonno R, Benestad SL (2021) Chronic wasting disease in Europe: new strains on the horizon. Acta Vet Scand 63:48

17. Nonno R, Di Bari MA, Pirisinu L, D'Agostino C, Vanni I, Chiappini B, Marcon S, Riccardi G, Tran L, Vikøren T, Våge J, Madslien K, Mitchell G, Telling GC, Benestad SL, Agrimi U (2020) Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America. Proc Natl Acad Sci USA 117:31417–31426

18. Pritzkow S, Gorski D, Ramirez F, Telling GC, Benestad SL, Soto C (2022) North American and Norwegian chronic wasting disease prions exhibit different potential for interspecies transmission and zoonotic risk. J Infect Dis 225:542–551

19. Béringue V, Vilotte JL, Laude H (2008) Prion agent diversity and species barrier. Vet Res 39:47

20. Kurt TD, Sigurdson CJ (2016) Cross-species transmission of CWD prions. Prion 10(1):83–91

21. Cullingham CI, Peery RM, Dao A, McKenzie DI, Coltman DW (2020) Predicting the spread-risk potential of chronic wasting disease to sympatric ungulate species. Prion 14:56–66

22. Tranulis MA, Tryland M (2023) The zoonotic potential of chronic wasting disease - a review. Foods 12:824

23. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock CJ (1997) Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 389:498–501

24. Tranulis MA (2002) Influence of the prion protein gene, *Prnp*, on scrapie susceptibility in sheep. APMIS 110:33–43

25. O'Rourke KI, Spraker TR, Hamburg LK, Besser TE, Brayton KA, Knowles DP (2004) Polymorphisms in the prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer. J Gen Virol 85:1339–1346

26. Belt PB, Muileman IH, Schreuder BE, Bos-de Ruijter J, Gielkens AL, Smits MA (1995) Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. J Gen Virol 76:509–517

27. González L, Pitarch JL, Martin S, Thurston L, Simmons H, Acín C, Jeffrey M (2014) Influence of polymorphisms in the prion protein gene on the pathogenesis and neuropathological phenotype of sheep scrapie after oral infection. J Comp Pathol 150:57–70

28. Collinge J, Clarke AR (2007) A general model of prion strains and their pathogenicity. Science 318:930–936

29. Peden AH, Suleiman S, Barria MA (2021) Understanding intra-species and inter-species prion conversion and zoonotic potential using protein misfolding cyclic amplification. Front Aging Neurosci 13:716452

30. Hansen H, Kapperud G, Mysterud A, Solberg EJ, Strand O, Tranulis M, Ytrehus B, Asmyhr M, Grahek-Ogden D (2017) CWD in Norway—a state of emergency for the future of cervids (Phase II). Norwegian Scientific Committee for Food Safety (VKM) https://vkm.no/download/18.645b8 40415d03a2fe8f253c5/1509532944615/CWD%20in%20Norway%20% E2%80%93%20a%20state%20of%20emergency%20for%20the%20fut ure%20of%20cervids%20(Phase%20II).pdf Accessed 28 June 2023

31. Harpaz E, Salvesen Ø, Rauset GR, Mahmood A, Tran L, Ytrehus B, Benestad SL, Tranulis MA, Espenes A, Ersdal C (2022) No evidence of uptake or propagation of reindeer CWD prions in environmentally exposed sheep. Acta Vet Scand 64:13

32. Ytrehus B, Grahek-Ogden D, Strand O, Tranulis M, Mysterud A, Aspholm M, Jore S, Kapperud G, Møretrø T, Nesbakken T, Robertson L, Melby K, Skjerdal T (2018) Factors that can contribute to spread of CWD—an update on the situation in Nordfjella, Norway Norwegian Scientific Committee for Food Safety (VKM) https://vkm.no/download/18.69622 9a71677d983532c0c11/1547126741061/CWD%20factors%20for% 20spread%202018.pdf Accessed 28 June 2023

33. Sigurdson CJ, Mathiason CK, Perrott MR, Eliason GA, Spraker TR, Glatzel M, Manco G, Bartz JC, Miller MW, Hoover EA (2008) Experimental chronic wasting disease (CWD) in the ferret. J Comp Pathol 138:189–196

34. Race B, Williams K, Orrú CD, Hughson AG, Lubke L, Chesebro B (2018) Lack of transmission of chronic wasting disease to cynomolgus macaques. J Virol 92:e00550-e618

35. Moore SJ, Smith JD, Richt JA, Greenlee JJ (2019) Raccoons accumulate PrP^{Sc} after intracranial inoculation of the agents of chronic wasting disease or transmissible mink encephalopathy but not atypical scrapie. J Vet Diagn Invest 31:200–209

36. Moore SJ, West Greenlee MH, Kondru N, Manne S, Smith JD, Kunkle RA, Kanthasamy A, Greenlee JJ (2017) Experimental Transmission of the chronic wasting disease agent to swine after oral or intracranial inoculation. J Virol 91:e00926-e1017

37. Hamir AN, Kunkle RA, Cutlip RC, Miller JM, Williams ES, Richt JA (2006) Transmission of chronic wasting disease of mule deer to Suffolk sheep following intracerebral inoculation. J Vet Diagn Invest 18:558–565

38. Wadsworth JDF, Joiner S, Linehan JM, Jack K, Al-Doujaily H, Costa H, Ingold T, Taema M, Zhang F, Sandberg MK, Brandner S, Tran L, Vikøren T, Våge J, Madslien K, Ytrehus B, Benestad SL, Asante EA, Collinge J (2021) Humanised transgenic mice are resistant to chronic wasting disease prions from Norwegian reindeer and moose. J Infect Dis 226:933–937

39. Bian J, Kim S, Kane SJ, Crowell J, Sun JL, Christiansen J, Saijo E, Moreno JA, DiLisio J, Burnett E, Pritzkow S, Gorski D, Soto C, Kreeger TJ, Balachandran A, Mitchell G, Miller MW, Nonno R, Vikøren T, Våge J, Madslien K, Tran L, Vuong TT, Benestad SL, Telling GC (2021) Adaptive selection of a prion strain conformer corresponding to established North American CWD during propagation of novel emergent Norwegian strains in mice expressing elk or deer prion protein. PLoS Pathog 17:e1009748

40. Saborio GP, Permanne B, Soto C (2001) Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 411:810–813

41. Salvesen Ø, Reiten MR, Espenes A, Bakkebø MK, Tranulis MA, Ersdal C (2017) LPS-induced systemic inflammation reveals an immunomodulatory role for the prion protein at the blood-brain interface. J Neuroinflammation 14:106

42. Meling S, Bårdsen K, Ulvund MJ (2012) Presence of an acute phase response in sheep with clinical classical scrapie. BMC Vet Res 8:113

43. Reetablering av villrein i Nordfjella er komplisert https://www.hjortevilt. no/reetablering-av-villrein-i-nordfjella-er-komplisert (in Norwegian). Accessed 25 June 2023

44. Skrantesjuke—forskrifter og regelverk https://www.hjortevilt.no/skran tesjuke-forskrifter-regelverk (in Norwegian). Accessed 25 June 2023

45. Cassmann ED, Frese RD, Greenlee JJ (2021) Second passage of chronic wasting disease of mule deer to sheep by intracranial inoculation compared to classical scrapie. J Vet Diagn Invest 33:711–720

46. Cassmann ED, Moore SJ, Greenlee JJ (2021) Experimental oronasal transmission of chronic wasting disease agent from white-tailed deer to Suffolk sheep. Emerg Infect Dis 27:3156–3158

47. Salvesen Ø, Espenes A, Reiten MR, Vuong TT, Malachin G, Tran L, Andréoletti O, Olsaker I, Benestad SL, Tranulis MA, Ersdal C (2020) Goats naturally devoid of PrP^C are resistant to scrapie. Vet Res 51:1

48. Rivrud IM, Bischof R, Meisingset EL, Zimmermann B, Loe LE, Mysterud A (2016) Leave before it's too late: anthropogenic and environmental triggers of autumn migration in a hunted ungulate population. Ecology 97:1058–1068

49. van Moorter B, Bunnefeld N, Panzacchi M, Rolandsen CM, Solberg EJ, Sæther B-E (2013) Understanding scales of movement: animals ride waves and ripples of environmental change. J Anim Eco 82:770–780

50. Mysterud A, Bischof R, Loe LE, Odden J, Linnell JDC (2012) Contrasting migration tendencies of sympatric red deer and roe deer suggest multiple causes of migration in ungulates. Ecosphere. https://doi.org/10.1890/ES12-00177.1

51. Robinson AL, Williamson H, Güere ME, Tharaldsen H, Baker K, Smith SL, Pérez-Espona S, Krojerová-Prokešová J, Pemberton JM, Goldmann W, Houston F (2019) Variation in the prion protein gene (*PRNP*) sequence of wild deer in Great Britain and mainland Europe. Vet Res 50:59

52. Schwabenlander MD, Culhane MR, Hall SM, Goyal SM, Anderson PL, Carstensen M, Wells SJ, Slade WB, Armién AG (2013) A case of chronic wasting disease in a captive red deer (*Cervus elaphus*). J Vet Diagn Invest 25:573–576

53. Moazami-Goudarzi K, Andréoletti O, Vilotte J-L, Béringue V (2021) Review on *PRNP* genetics and susceptibility to chronic wasting disease of Cervidae. Vet Res 52:128

54. Balachandran A, Harrington NP, Algire J, Soutyrine A, Spraker TR, Jeffrey M, González L, O'Rourke KI (2010) Experimental oral transmission of chronic wasting disease to red deer (*Cervus elaphus elaphus*): early detection and late stage distribution of protease-resistant prion protein. Can Vet J 51:169–178

55. Wik L, Mikko S, Klingeborn M, Stéen M, Simonsson M, Linné T (2012) Polymorphisms and variants in the prion protein sequence of European moose (*Alces alces*), reindeer (*Rangifer tarandus),* roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) in Scandinavia. Prion 6:256–260

56. Güere ME, Våge J, Tharaldsen H, Kvie KS, Bårdsen BJ, Benestad SL, Vikøren T, Madslien K, Rolandsen CM, Tranulis MA, Røed KH (2022) Chronic wasting disease in Norway-A survey of prion protein gene variation among cervids. Transbound Emerg Dis 69:e20–e31

57. Zerr I (2022) (2022) Prion 2022 Conference abstracts: pushing the boundaries. Prion 16:95–253

58. Våge J, Hopp P, Vikøren T, Madslien K, Tarpai A, Moldal T, Benestad SL (2021) The surveillance programme for Chronic Wasting Disease (CWD) in free-ranging and captive cervids in Norway 2021 https://www.vetinst.no/overvaking/chronic-wasting-disease-vilt-cwd Accessed 28 June 2023

59. Güere ME, Våge J, Tharaldsen H, Benestad SL, Vikøren T, Madslien K, Hopp P, Rolandsen CM, Røed KH, Tranulis MA (2020) Chronic wasting disease associated with prion protein gene (*PRNP*) variation in Norwegian wild reindeer (*Rangifer tarandus*). Prion 14:1–10

60. Bélondrade M, Nicot S, Mayran C, Bruyere-Ostells L, Almela F, Di Bari MA, Levavasseur E, Watts JC, Fournier-Wirth C, Lehmann S, Haïk S, Nonno R, Bougard D (2021) Sensitive protein misfolding cyclic amplification of sporadic Creutzfeldt-Jakob disease prions is strongly seed and substrate dependent. Sci Rep 11:4058

Publisher's Note