Norwegian University of Life Sciences
Faculty of Veterinary Medicine
Department of Preclinical Sciences and Pathology

Philosophiae Doctor (PhD)
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Genetic variation in Norwegian cervids – relevance to the occurrence of Chronic Wasting Disease (CWD)

Genetisk variasjon hos norske hjortedyr – relevans for forekomst av skrantesyke

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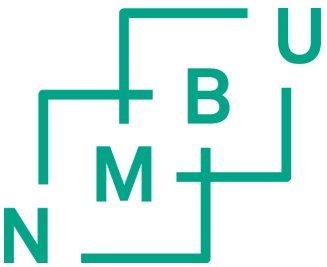

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Oslo, June 2021

Mariella Evelyn Güere Calderón

Abbreviations and acronyms

Standardized names for genes are assigned according to current guidelines of the Human Genome Organization (HUGO) Gene Nomenclature Committee (HGNC) (Bruford et al. 2020).

BSE	Bovine Spongiform Encephalopathy
CA DIST	distance between alpha-carbons
CJD	Creutzfeldt-Jakob disease
CNS	Central nervous system
CWD	Chronic Wasting Disease
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
HGVS	Human Genome Variation Society
HWE	Hardy-Weinberg equilibrium
IHC	immunohistochemistry
IUPAC-IUB	International Union of Pure and Applied Chemistry and IUB; now the International Union of Biochemistry and Molecular Biology, IUBMB
PCR	polymerase chain reaction
PRND	downstream prion protein-like gene; now prion like protein doppel gene
PRNP	prion protein gene
PRNPψ	prion protein pseudogene
PrP^{C}	cellular prion protein
PrP^{Sc}	protein associated with prion disease
NMR	nuclear magnetic resonance
MBM	meat and bone meal
NHF	*Norsk Hjorteavlsforening*
OIE	*Office International des Epizooties*; now the World Organisation for Animal Health
RT-QuIC	real-time quaking-induced conversion
sCJD	Sporadic Creutzfeldt-Jakob Disease
SNV	single nucleotide variant; replaces single nucleotide polymorphism, SNP

STRIDE	STRuctural IDEntification
SPRN	shadow of prion protein gene
TSE	transmissible spongiform encephalopathy
vCJD	Variant Creutzfeldt-Jakob Disease
WAHIS-Wild	World Animal Health Information System for wildlife diseases
VKM	*Vitenskapskomiteen for mattrygghet*
WB	western blot

Summary

The etiological agents' nature has a pivotal role in the pathogenesis of infectious diseases. Prion diseases are unique in having a misfolded protein (PrP^{Sc}) as the sole transmissible agent. The host cellular protein (PrP^{C}) is the substrate for misfolding and aggregation by PrP^{Sc}. Variability in the prion protein gene (*PRNP*) encoding PrP^{C} affects prion disease progression and susceptibility, probably by inducing changes that may alter the efficiency of a pathogenic PrP^{C}-to-PrP^{Sc} conversion. This principle applies to Chronic Wasting Disease (CWD; MONDO_0002680), a prion disease affecting members of the family *Cervidae*. CWD is widely distributed in North America from where it was imported to the Republic of Korea. Identification of CWD in Norway in wild reindeer (*Rangifer tarandus*) in 2016 and its subsequent identification in moose (*Alces alces*), and red deer (*Cervus elaphus*) has raised questions regarding the apparent geographical expansion of this disease in Europe. One key question targets the variation in the host *PRNP*, an important genetic risk factor in prion disease.

This thesis studies *PRNP* variability in Norwegian cervids, where most European cases have been identified to June 2021. The endemic Norwegian species are reindeer, red deer, moose, and roe deer (*Capreolus capreolus*) with reindeer managed between, semi-domestic- or wild- populations. The first cases of CWD in Europe were recorded among the Norwegian wild reindeer population in Nordfjella 1, part of the Nordfjella management area in Southern Norway, where 19 CWD cases were identified. In this population, the risk to test positive to CWD was found to be greater in adult males and this likelihood increased with age. Similarly, two *PRNP* genotypes were found to increase the risk of CWD development in these wild reindeer. These susceptible genotypes combine two variants of *PRNP* (called alleles) named *226Q* and *deletion_226Q*.

Aware of the implications further spread of CWD could have for European cervid populations, a collaboration between scientists from Great Britain, the Czech Republic and Norway allowed to analyze the spatial variation of *PRNP* alleles across Europe. Using red deer as a proxy to monitor such variability, significant *PRNP* variation between countries and subregions was observed. The CWD susceptible allele *226Q* was found in all European red deer subpopulations, although at variable frequencies.

Spatial variation in *PRNP* alleles' distribution was also observed within Norwegian populations of reindeer, red deer, and moose, but not roe deer, which was

monomorphic. All species harbored the allele *226Q*, but higher frequencies were found in reindeer, red deer, and moose in certain areas. The distribution of *PRNP* alleles differed between wild and semi-domesticated reindeer. Alleles associated with high susceptibility to CWD were identified in >55% of wild reindeer and <20% of semi-domesticated reindeer. This may reflect the different origins of the populations and/or selection processes during domestication and breeding.

Norwegian and British cervids of other species sharing a genotype (*226Q*/*226Q*) associated with an increased risk of contagious CWD, as reported in Norwegian reindeer, implies that a considerable proportion of these populations would be susceptible to CWD. Further research on the *PRNP* alleles identified in European cervids would be beneficial to understand their impact in animal physiology and CWD dynamics.

Sammendrag

Ved infeksiøse sykdommer har egenskapene til agens en sentral plass i patogenesen. Unikt for prionsykdommer er at det infeksiøse agens er et protein. Vertsorganismens cellulære prion protein (PrP^{C}) er substratet som kan endre seg og gi mulighet for opphopning av feilfoldet protein (PrP^{Sc}). Variasjon i genet, *PRNP*, som koder for PrP^{C} påvirker sykdomsutvikling og mottakelighet ved prionsykdom. Sannsynlig mekanisme er at den genetiske variasjonen påvirker effektivitet i omdannelsen av PrP^{C} til PrP^{Sc}. Dette prinsippet gjelder for Chronic Wasting Disease (CWD, også kalt skrantesjuke; MONDO_0002680). Dette er en prionsykdom som rammer dyr i hjortedyrfamilien. CWD er vidt utbredt i Nord-Amerika, og ble eksportert med livdyr fra Canada til Sør-Korea. Påvisningen av CWD hos norsk villrein (*Rangifer tarandus*) i 2016, med etterfølgende funn hos elg (*Alces alces*) og hjort (*Cervus elaphus*), har reist spørsmål om geografisk spredning av sykdommen i Europa. En viktig risikofaktor vil være variasjonen i *PRNP* hos bestandene som utsettes for denne prionsykdommen.

Denne doktorgraden studerer variasjon i *PRNP* hos norske hjortedyr, som er de bestandene i Europa der flest tilfeller av sykdommen er påvist fram til juni 2021. Norge har ville bestander av reinsdyr, hjort, elg og rådyr (*Capreolus capreolus*). I tillegg er det også tamreindrift som er organisert i ulike reinbeitedistrikter. Den første påvisningen av CWD i Europa ble gjort hos en villrein i Nordfjella villreinområde (Nordfjella sone 1). Totalt ble det avdekket 19 tilfeller i denne bestanden. I dette studiet ble det identifisert en forhøyet CWD-risiko for voksen bukk, en risiko som økte med økende alder. Det ble også avdekket to *PRNP* genotyper som ga økt risiko for CWD. Disse genotypene er en kombinasjon av to varianter av *PRNP* (såkalte alleler) som er gitt betegnelsene *226Q* og *deletion_226Q*.

For bedre å forstå spredingspotensialet av CWD til hjortedyrbeatanden i Europa, ble det inngått et samarbeid mellom forskere fra Storbritannia, Tsjekkia og Norge. Samarbeidet ga mulighet til å undersøke romlig fordeling av *PRNP*-alleler i Europa. Ved bruk av hjort som modell ble det avdekket signifikant variasjon mellom land og regioner. Allel *226Q*, som hos villrein er identifisert som et risikoallel, ble funnet i alle europeiske hjortebestander, dog i ulike frekvenser.

Romlig fordeling av *PRNP*-alleler ble også observert hos norsk reinsdyr, hjort og elg, men ikke hos rådyr som var uten *PRNP* variasjon. Alle arter hadde allele *226Q* med betydelig geografiske variasjoner hos reinsdyr, hjort og elg. Fordelingen av *PRNP*-alleler var ulik mellom villrein og tamrein, og alleler definert med høy CWD risiko

opptrådte i >55 % av undersøkte villrein mot <20 % i tamrein. Dette mønsteret kan gjenspeile ulik opprinnelse av bestandene og/eller seleksjonsprosesser knyttet til domestisering og avl.

PRNP variasjonen hos norske og britiske hjortedyr, som deler risiko-genotypen for smittsom CWD hos reinsdyr (*226Q/226Q*), antyder at store deler av bestandene vil være mottagelige for sykdommen. For å bedre forstå betydningen av de avdekkede *PRNP*-variantene i Europa, og deres virkning på sykdomsdynamikk og dyrenes fysiologi, kreves ytterligere undersøkelser og forskning.

Resumen

La naturaleza de los agentes etiológicos tiene un papel fundamental en la patogénesis de las enfermedades infecciosas. Las enfermedades causadas por priones tienen la particularidad de tener una proteína mal plegada (PrP^{Sc}) como único agente transmisible. La proteína celular del huésped (PrP^{C}) es el sustrato para el mal plegamiento y la agregación por parte de PrP^{Sc}. La variabilidad en el gen de la proteína priónica (*PRNP*), que codifica la PrP^{C}, afecta a la progresión de la enfermedad priónica y a la susceptibilidad, probablemente induciendo cambios que pueden alterar la eficiencia de la conversión patogénica de PrP^{C} en PrP^{Sc}. Este principio se aplica a la caquexia crónica (CWD por su nombre en inglés, *Chronic Wasting Disease*; MONDO_0002680), una enfermedad priónica que afecta a miembros de la familia *Cervidae*. La caquexia crónica está ampliamente distribuida en América del Norte, de donde fue importada a la República de Corea. La identificación de la caquexia crónica en Noruega en renos salvajes (*Rangifer tarandus*) en 2016 y su posterior identificación en alces (*Alces alces*) y un ciervo rojo (*Cervus elaphus*) ha planteado preguntas sobre la aparente expansión geográfica de esta enfermedad en Europa. Una de las preguntas clave se centra en la variación del *PRNP* del huésped, un importante factor de riesgo genético en la enfermedad priónica.

Esta tesis estudia la variabilidad del gen *PRNP* en los cérvidos noruegos, donde se han identificado la mayoría de los casos europeos hasta junio del 2021. Las especies noruegas endémicas son el reno, el ciervo rojo, el alce y el corzo (*Capreolus capreolus*), con renos manejados como poblaciones semidomésticas o salvajes. Los primeros casos de caquexia crónica en Europa se registraron entre la población noruega de renos salvajes en Nordfjella 1, parte del área de gestión de Nordfjella, en el sur de Noruega, donde se identificaron 19 casos de caquexia crónica. En esta población, el riesgo de dar positivo a la caquexia crónica resultó ser mayor en los machos adultos y esta probabilidad aumentó con la edad. Asimismo, se descubrió que dos genotipos *PRNP* aumentan el riesgo de desarrollo de la caquexia crónica en estos renos salvajes. Estos genotipos susceptibles combinan dos variantes del gen *PRNP* (denominadas alelos) denominadas *226Q* y *deletion_226Q*.

Conscientes de las implicaciones que podría tener una mayor propagación de la caquexia crónica para las poblaciones europeas de cérvidos, una colaboración entre científicos de Gran Bretaña, la República Checa y Noruega permitió analizar la variación espacial de los alelos del *PRNP* en Europa. Utilizando el ciervo rojo como

representante para monitorear dicha variabilidad, se observó una importante variación del gen *PRNP* entre países y subregiones. El alelo *226Q*, susceptible a la caquexia crónica, se encontró en todas las subpoblaciones europeas de ciervos rojos, aunque con frecuencias variables.

También se observó una variación espacial en la distribución de alelos del gen *PRNP* en las poblaciones noruegas de renos, ciervos rojos y alces, pero no en la de corzos, que era monomórfica. Todas las especies albergaban el alelo *226Q*, pero se encontraron frecuencias más altas en renos, ciervos rojos y alces en determinadas zonas. La distribución de los alelos del gen *PRNP* difería entre los renos salvajes y los semidomesticados. Los alelos asociados a una alta susceptibilidad a la caquexia crónica se identificaron en >55% de los renos salvajes y en <20% de los renos semidomesticados. Esto puede reflejar los diferentes orígenes de las poblaciones y/o los procesos de selección durante la domesticación y la cría.

El hecho de que los cérvidos noruegos y británicos de otras especies que comparten un genotipo (*226Q*/*226Q*) asociado a un mayor riesgo de la caquexia crónica contagiosa, como se ha reportado en los renos noruegos, implica que una proporción considerable de estas poblaciones sería susceptible de contraer la caquexia crónica. Sería beneficioso seguir investigando los alelos del gen *PRNP* identificados en los cérvidos europeos para comprender su impacto en la fisiología de los animales y la dinámica de la caquexia crónica.

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1 List of articles

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II. **Mariella E. Güere**, Jørn Våge, Helene Tharaldsen, Sylvie L. Benestad, Turid Vikøren, Knut Madslien, Petter Hopp, Christer M. Rolandsen, Knut H. Røed & Michael A. Tranulis (2020). Chronic wasting disease associated with prion protein gene (*PRNP*) variation in Norwegian wild reindeer (*Rangifer tarandus*). *Prion*, 14:1, 1-10. https://doi.org/10.1080/19336896.2019.1702446

III. Amy L. Robinson, Helen Williamson, **Mariella E. Güere**, Helene Tharaldsen, Karis Baker, Stephanie L. Smith, Sílvia Pérez-Espona, Jarmila Krojerová-Prokešová, Josephine M. Pemberton, Wilfred Goldmann, Fiona Houston (2019). Variation in the prion protein gene (*PRNP*) sequence of wild deer in Great Britain and mainland Europe. *Veterinary Research* 50, 59. https://doi.org/10.1186/s13567-019-0675-6

IV. **Mariella E. Güere**, Jørn Våge, Helene Tharaldsen, Kjersti S. Kvie, Bård-Jørgen Bårdsen, Sylvie L. Benestad, Turid Vikøren, Knut Madslien, Christer M. Rolandsen, Michael A. Tranulis, Knut H. Røed (2021). Chronic wasting disease in Norway - a survey of prion protein gene variation among cervids. Submitted to *Transboundary and Emerging Diseases*.

2 Introduction

2.1 Prion diseases

Prion diseases are incurable neurodegenerative diseases affecting humans and animals (Rivera et al. 2019) (**Table 1**). The diseases are caused by prions (PrP^{Sc}) which are misfolded and aggregation prone isoforms of the prion protein (PrP^{C}), a neuroprotective host glycoprotein (Linsenmeier et al. 2017). Interaction between PrP^{Sc} and PrP^{C} can induce a pathogenic PrP^{C}-to-PrP^{Sc} conversion (Aguzzi et al. 2008). In other words: PrP^{Sc} acts as a "seeding template" and PrP^{C} as a "substrate", which in a poorly understood process, is converted into novel PrP^{Sc} seeds that can misfold further PrP^{C} molecules, thereby propagating the prion.

Accumulation of PrP^{Sc} within and around neurons is strongly associated with a spongiform neurodegeneration which is why prion diseases were first referred to as transmissible spongiform encephalopathies (TSEs). Whereas the term TSE nicely summarizes the main features of a prion disease, the modes of PrP^{Sc} transmission can be broad and influence the epidemiology of the disease. In many prion diseases, PrP^{Sc} accumulations are observed in organs outside the central nervous system (CNS), including the spleen, tonsils, retro-pharyngeal lymph nodes and submucosal lymphoid tissues of the gastrointestinal tract (van Keulen et al. 1996, Sigurdson et al. 1999). Aggregates of PrP^{Sc} in these organs can significantly precede neuroinvasion and appear to be tolerated i.e. without recognizable organ dysfunction. Detection of PrP^{Sc} aggregates in peripheral lymphoid tissues is sufficient for diagnosis of prion disease, even in the absence of such aggregates in the CNS (Sigurdson et al. 1999). Based on epidemiological data, human prion diseases are classified as familial (inherited), sporadic, and acquired (Gambetti et al. 2003), a classification partly applicable to animals. For instance, scrapie in sheep (Benestad et al. 2003) and BSE in cattle (Martucci et al. 2009) are categorized into classical and atypical forms, corresponding to acquired (Scheckel and Aguzzi 2018) and sporadic (Curcio et al. 2016) forms in humans. Epidemiological classification is a reflection of complex host, pathogen, and environment interactions. Deciphering the framework of the epidemiological triad (Vander Wal et al. 2014) for prion diseases is important, not least to enable decision-makers to develop efficient policies to limit or stop further dissemination of these diseases.

Table 1. *Overview of animal and human prion diseases. MBM: meat and bone meal.* ***Table 1*** *is a derivative of "Table I" by Rivera et al. (2019) (https://doi.org/10.2147/VMRR.S197404) used under CC BY-NC 3.0 with comments on atypical prion diseases in livestock from Tranulis et al. (2011).*

Host	Prion Disease Name	Report	Affected mammals	Mode of Natural Transmission
Cervidae	Chronic Wasting Disease (CWD)	1967	Elk (wapiti), mule deer, white-tailed deer, moose, caribou/ reindeer (*Rangifer*), red deer, sika deer, reindeer, axis deer, roe deer, fallow deer, Muntjac	Horizontal transmission via direct contact with CWD infected cervids or indirectly via contact with CWD contaminated plants or inanimate fomites (e.g., soil, mineral licks, plastic reservoirs, etc.) Vertical transmission from mother to offspring *in utero*
	Scrapie	1732	Sheep, goats	Horizontal and vertical (same as CWD)
	Nor98/atypical scrapie	1998	Sheep, goats	Unknown etiology (occurs spontaneously)
Bovidae	Bovine Spongiform Encephalopathy (BSE)	1986	Cattle	Exposure to contaminated TSE tissue in food (MBM)
	Atypical Low-type (BSE-L) Atypical Hight type (BSE-H)	2004	Cattle	Unknown etiology (occurs spontaneously)
Hominidae	Sporadic Creutzfeldt-Jakob Disease (sCJD)	1920	Humans	Unknown etiology (occurs spontaneously)
	Familial Creutzfeldt-Jakob Disease	1924	Humans	Familial prion diseases (inherited)
	Gerstmann-Sträussler-Scheinker Syndrome	1936	Humans	Familial prion diseases (inherited)
	Kuru	1957	Humans	Exposure during cannibalistic funeral rituals to prion contaminated tissue (brain) (acquired)
	Iatrogenic Creutzfeldt-Jakob Disease (iCJD)	1974	Humans	Exposure during surgery to CJD-contaminated instruments or via organ and tissue transplant (acquired)
	Fatal Familial Insomnia	1986	Humans	Familial prion diseases (inherited)
	Variant Creutzfeldt-Jakob Disease (vCJD)	1996	Humans	Exposure via ingestion of BSE-contaminated food (acquired)

Host	Prion Disease Name	Report	Affected mammals	Mode of Natural Transmission
Mustelidae	Transmissible Mink Encephalopathy	1965	Mink	Exposure to contaminated TSE tissue in food (acquired)
Zoo Animals	Ungulate Spongiform Encephalopathy	1988	In zoo animals including members of the family *Bovidae*	Exposure via ingestion of BSE-contaminated food (MBM)
Felidae	Feline Spongiform Encephalopathy	1990	Housecats and captive wild cats	Identified in domesticated and captive wild cats exposed to BSE-contaminated food (bovine tissue or meat and MBM)
Camelidae	Camel Prion Disease	2015	Dromedary camels	Unknown etiology

2.1.1 Pathogenic conversion and species barriers

PrP^{Sc} deposition and associated histopathologically visible vacuolation in the central nervous system (CNS) are pathognomonic of prion diseases. PrP^{Sc} identification alone is an earlier diagnostic biomarker compared to identification of brain lesions (Benestad and Telling 2018). An understanding of prion biology is fundamental to further understanding the agent, its relationship with the host and the underlying epidemiology of prion diseases. From this point, focus will be placed on scrapie affecting sheep, the prototype of prion diseases (**Table 1**) which have provided the first clues on the prion biology.

Scrapie was recorded as early as 1732 in Great Britain (McGowan 1922) and scrapie diagnoses were for many years subject to stigma and secrecy in Western Europe. It took almost 200 years before the scientific community openly discussed scrapie, allowing identification of its unusually prolonged incubation (Stockman 1913) and experimental confirmation of its transmissibility from diseased to healthy animals (Cuillé et al. 1936). The scrapie agent was originally considered as a "slow virus" (Sigurdsson 1954). This association was also proposed for Kuru – based on its resemblance to scrapie – (Hadlow 1959) and for Creutzfeldt-Jakob Disease (CJD) given its similarities to Kuru (Klatzo et al. 1959). The successful transmission of Kuru and CJD to chimpanzees (Gajdusek et al. 1966, Gibbs et al. 1968), previously demonstrated for scrapie between sheep, reaffirmed a common mechanism in these diseases. Bioassays of the scrapie agent in rodents allowed for replication and improved description of the scrapie agent's properties, allowing more effective purification of the agent (Prusiner 1998).

The scrapie agent, regarded as a "" slow virus", proved resistant to procedures that alter nucleic acids but not proteins (Latarjet et al. 1970, Prusiner 1982). Paradoxically, the scrapie agent was sensitive to procedures that either modify or denature proteins while maintaining biologically active nucleic acids (Prusiner 1982). In this way, Prusiner (1982) demonstrated the protein composition of the scrapie agent and coined the term "prion" that stands for *pro*teinaceous *in*fectious particle.

Further purification of the scrapie agent yielded a protein with a molecular mass of 27000 to 30000 Daltons (PrP 27-30) more resistant to digestion by proteinase K than proteins of similar molecular mass normally found in the brain (Bolton et al. 1982). This protein is the protease-resistant core of PrP^{Sc}, which (following amino acid sequencing by Edman degradation (Prusiner et al. 1984)), allowed generation of oligonucleotides that enabled identification of incomplete PrP cDNA clones in

scrapie-infected- and normal- brains of hamster (Oesch et al. 1985) and mice (Chesebro et al. 1985). Shortly after, almost complete cDNAs encoding the entire open reading frame (ORF) of PrP was recovered (Basler et al. 1986). It was shown that the same chromosomal gene (the prion protein gene or *PRNP*) encoded the scrapie isoform of PrP (PrP^{Sc}) and a soluble and proteinase sensitive isoform that was designated "cellular prion protein" (PrP^{C}) (Basler et al. 1986)(Basler et al 1986).

Subsequently, PrP^{C} was found to be essential for the pathogenesis of scrapie (Büeler et al. 1993, Prusiner et al. 1993, Sailer et al. 1994). In other words, PrP^{Sc} alone does not induce neurodegeneration (Brandner et al. 1996) and neuronal expression of PrP^{C} is necessary for prion disease pathology to develop (Mallucci et al. 2003). The absolute requirement for PrP^{C} for prion disease development has also recently been demonstrated in goats lacking PrP^{C} due to a naturally occurring substitution in *PRNP* that terminates the translation at codon 32 (Salvesen et al. 2020). The molecular details of how PrP^{C} and PrP^{Sc} interplay elicits neurodegeneration remain poorly understood.

From early attempts at experimental transmission, Pattison (1965) noted that when donor and recipient belonged to different species, the interval between exposure and onset of disease was lengthier than when donor and recipient were of the same species, a phenomenon referred to as "species barrier". One factor contributing to the "species barrier" is the difference in PrP amino acid sequence between donor and recipient, which are respectively determined by donor and recipient *PRNP* coding sequence (Prusiner 1998). However, transmission is facilitated if both share the same primary PrP^{C} structure (Prusiner et al. 1990). The variance in disease onset attributed to *PRNP* differences following interspecies transmission was also identified in murine lineages infected with scrapie PrP^{Sc}, which distinctly displayed short or long incubation (Carlson et al. 1989). These intervals were ascribed to distinct PrP^{Sc} allotypes[1] differing by two amino acids.

Beyond the experimental settings, Hsiao et al. (1989) reported a substitution in *PRNP* to be associated with development of a rare human prion disease. Subsequent investigations in naturally occurring prion diseases in sheep, humans and various other species have underscored the importance of *PRNP* variation for disease development – see reviews by Robinson et al. (2012) and Colby and Prusiner (2011). Despite the possible contribution of as-yet unknown genetic determinants (Lukic and Mead 2011, Mead et al. 2011), *"All roads lead to Rome"* and *PRNP* variability of the

[1] Protein variants that are genetically determined

host has been repetitively proven to be a key determinant, not only for onset of disease but also for various nuances in prion disease pathology.

2.1.2 CWD: Chronic wasting disease

Among the various prion diseases (**Table 1**), two transmit horizontally under normal circumstances and both are observed in ruminants. One is classical scrapie, the prototypical prion disease first described in the 1700s and intensively studied for almost 100 years. The other is Chronic Wasting Disease (CWD) affecting cervids, a relatively new and proliferating disease. CWD is distinguished from other prion diseases by affecting both wild and farmed animals, by its expanding geographical range and increasing number of host species (Benestad and Telling 2018).

By the end of the 1970's, Williams and Young (1980) had observed for almost a decade a "chronic wasting disease" in captive mule deer (*Odocoileus hemionus hemionus*). They concluded that the disease was a TSE on the basis of comparative brain histopathology from diseased animals, which was an approach also used to define Kuru and CJD in the 1960's (Hadlow 1959, Klatzo et al. 1959). Shortly after, elk (wapiti, *Cervus canadensis*) from the very same facilities in Wyoming and Colorado, (USA) were also diagnosed with CWD (Williams and Young 1982). The animals concerned had had occasional, direct or indirect contact with the infected deer reported by Williams and Young (1980). During the following decade, CWD was identified in free-ranging (wild) cervids in Colorado, including another host, the white-tailed deer (*Odocoileus virginianus*) (Spraker et al. 1997). These early cases also included a black-tailed deer (*O. h. columbianus*) and a mule × white-tailed deer hybrid (Williams and Young 1980). Based on evidence at that time, it became evident that CWD could have the potential to be highly contagious and affect various *Cervidae* species.

By the 2000's, researchers became aware of the potential for spread of CWD amongst wild animals (Miller et al. 2000). In the words of CWD experts Williams and Miller (2002): *"Only three species, all members of the family Cervidae,…"* were *"known to be naturally susceptible to CWD, namely: mule deer, white-tailed deer and Rocky Mountain elk"* as published by the OIE (*Office International des Epizooties*, renamed as World Organisation for Animal Health). In this OIE publication from 2002, the experts also reported, that with the exception of some cases in Canada, *"no CWD-affected free-ranging cervids have been detected outside the contiguous Wyoming-Colorado-Nebraska endemic area"*. In other words, in 2002 CWD had a limited (known) geographic distribution with few naturally affected *Cervidae* species, whereas by June 2021 -the time this thesis is submitted-, CWD has been recognized further afield the

original endemic area and in other species within the *Cervidae* (in natural and experimental conditions).

Today, CWD has been reported from 26 states in the USA and three provinces of Canada[2]. Outside of North America, it has been identified in the Republic of Korea and in Fennoscandia[3] (Rivera et al. 2019). Moreover, naturally occurring CWD cases have been diagnosed in moose (*Alces alces*), red deer (*Cervus elaphus*), Sika deer (*Cervus nippon*) and reindeer (*Rangifer tarandus*) (Kreeger et al. 2006, Lee et al. 2013, Benestad et al. 2016, Vikøren et al. 2019, Walther et al. 2019). In this context, the OIE Working Group on Wildlife[4] has emphasized that further spread of CWD throughout Europe can potentially have major negative impacts on cervid populations and their management (OIE 2016). Accordingly, the OIE (2018) considers CWD to be an emerging and noteworthy wildlife disease in Fennoscandia and North America.

2.1.2.1 Epidemiology and risk factors

There is major interest in establishment of effective control of CWD through a better understanding of the distribution and determinants of the disease (**Figure 1**). Nonetheless, acquaintance with the epidemiology of CWD remains limited, despite an initiative from OIE encouraging member states to report CWD occurrence and relevant epidemiological data through WAHIS-Wild[5] (OIE 2017). The following section provides an up-to-date overview of CWD epidemiology as described in North America. Knowledge of CWD in Europe, is increasing, including that related to emergence of sporadic cases of CWD in moose (Pirisinu et al. 2018, Ågren et al. 2021), and will be independently discussed in section 2.5 (CWD in Europe, 2016 to 2020*).

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[2] https://www.usgs.gov/centers/nwhc/science/expanding-distribution-chronic-wasting-disease?qt-science_center_objects=0#qt-science_center_objects accessed on April 13th, 2021

[3] https://www.lexico.com/definition/fennoscandia

[4] https://www.oie.int/en/standard-setting/specialists-commissions-working-ad-hoc-groups/working-groups-reports/working-group-on-wildlife/ accessed on November 14th, 2020

[5] OIE's World Animal Health Information System for wildlife diseases considered to require surveillance; see https://www.oie.int/en/animal-health-in-the-world/wahis-portal-animal-health-data/

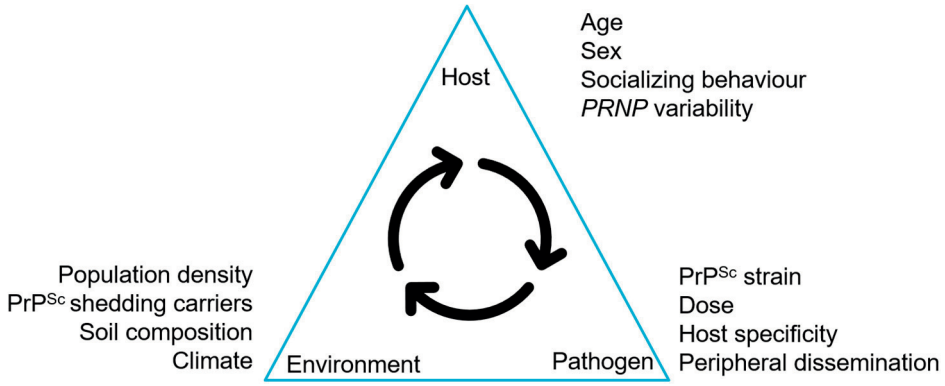

Figure 1. *The epidemiological triad of Chronic Wasting Disease. The triangle displays the interaction of host, pathogen, and environment, including known risk factors for the disease in cervids in North America. Refer to synopsis by Saunders et al. (2012).*

Wild and captive populations show differences in the disease frequency and distribution. CWD prevalence is influenced by animal density, evidenced by the high frequency observed in captive herds (Haley et al. 2019) compared to lower levels in wild populations (Manjerovic et al. 2014). Notably, CWD incidence increases slowly over time in the wild (Spraker et al. 1997, Rees et al. 2012, Manjerovic et al. 2014, DeVivo et al. 2017), but can increase more rapidly in captive held populations (Keane et al. 2008). On the other hand, the spread of CWD appears to be closely related to dispersal processes acting in the host (Cullingham et al. 2011, Wilkinson 2017). Whereas a progressive diffusion into neighboring areas is commonly observed in wild populations (Kelly et al. 2010, Rees et al. 2012), a jump dispersal, most likely human-mediated, has been observed in captive populations in areas without previous reports of CWD (Sohn et al. 2002, Kahn et al. 2004). Nonetheless, these processes are not mutually exclusive and may combine to result in the geographical expansion of CWD observed. This is illustrated by sequential or almost concurrent CWD emergence in captive populations and in nearby wild populations (Williams and Young 1980, Williams and Young 1982, Miller et al. 2000, Joly et al. 2006, Keane et al. 2008). Transmission of CWD, either directly between animals or indirectly by environmental contamination, depends on factors such as social behavior, habitat preferences and movement range (Schauber and Woolf 2003, Rees et al. 2012).

Mule deer, white-tailed deer and elk in which the majority of CWD cases have been reported, are wide ranging species (Ager et al. 2003, Cullingham et al. 2011), whereas cases in sympatric moose are few (Rivera et al. 2019). The rarity of CWD in moose is

possibly explained by the less social behavior of the species (Baeten et al. 2007) or minimal habitat overlap with other cervids in endemic CWD areas (Pirisinu et al. 2018). In addition, a decreasing CWD prevalence is observed in mule deer, white-tailed deer, and elk respectively (Spraker et al. 1997, Rees et al. 2012, DeVivo et al. 2017). This gradient may be at least partly explained by social grouping in these species. Interactions within kin social groups likely increase the risk of CWD transmission (Cullingham et al. 2010, Grear et al. 2010, Cullingham et al. 2011). Contact between kindred animals can be more frequent and intense than between non-related animals (Schauber and Woolf 2003). The three most affected species are known to be socially organized into matrilineal groups, allowing for more effective contact between animals, which are more stable in mule and white-tailed deer than in elk (Cullingham et al. 2011, Vander Wal et al. 2012). Non-kin interactions also occur in elk, and these may result in differences in CWD transmission compared to those influencing transmission in white-tailed deer and mule deer (Vander Wal et al. 2012). Similarly, the higher frequency of CWD in mule deer compared to sympatric white-tailed deer may relate to higher group stability and larger spatial coverage in mule deer allowing wider and more efficient transmission among kin matriarchic groups (Cullingham et al. 2011).

Despite host dispersal and social interaction as contributors to CWD transmission, exposure to PrP^{Sc} contaminated environments may result in transmission of CWD in the absence of animal-to-animal contact (Miller et al. 2006, Mathiason et al. 2009). This may gain prominence over time, with increasing and persistent contamination. Notably, CWD transmission, either by direct (animal-animal) or indirect (animal-environment-animals) contact, can be influenced by the innate susceptibility of the host population, which in turn is largely determined by *PRNP* variability (**Figure 1**).

PRNP variability and CWD status are clearly associated, a principle repeatedly observed in both wild and captive populations (O'Rourke et al. 2004, Johnson et al. 2006, Kelly et al. 2008, Brandt et al. 2015, Haley et al. 2020). In addition, *PRNP* variability appears to influence other aspects of CWD including disease progression and strain adaptation, while *PRNP* variation may occur across geographical space. *PRNP* variation will be discussed in section 2.3.

2.1.2.2 Livestock prion diseases and CWD – zoonotic potential

Monitoring of CWD by the OIE confirms its importance for wild animals and the need for early warning to protect human and livestock health[6]. The BSE crisis and subsequent vCJD appearance in 1996 in the United Kingdom illustrates this concern. The epidemiological evidence indicates that increased exposure to PrP^{Sc} through animal feed resulted in emergence of BSE in cattle (Wilesmith et al. 1991, Denny and Hueston 1997) and a subsequent zoonotic transmission (vCJD) (Bruce et al. 1997, Hill et al. 1997) via dietary exposure (Lasmézas et al. 2005). The BSE strain produces a characteristic pattern of disease in infected animals which was observed in mice inoculated with vCJD but not in sCJD (Bruce et al. 1997). Lesion profiles in mice inoculated with either vCJD or BSE inoculum were indistinguishable (Hill et al. 1997). This pathway illustrates an interspecies adaptation in PrP^{Sc}, with transmission to a new host (human) and manifestation of a new form of prion disease (vCJD). The scenario raises concerns on the possible implications of animals affected by prion diseases entering the animal feed or human food chain may have for public health (Wilesmith et al. 1991, Denny and Hueston 1997).

Like scrapie, CWD is a highly transmissible prion disease, with a host range currently limited to the family *Cervidae*. Infected hosts are known to shed PrP^{Sc} to the environment (Tamgüney et al. 2009, Plummer et al. 2017, Tennant et al. 2020). The subsequent environmental contamination raises concerns that livestock grazing these contaminated areas might develop a novel CWD-related prion disease (Benestad and Telling 2018) that could subsequently result in foodborne transmission to humans.

The likelihood that livestock can develop disease following exposure to CWD prions has been assessed by means of an intracerebral challenge model, which is the most efficient route of exposure despite being an unnatural transmission pathway (Hamir et al. 2007). The species barrier existing between domestic ruminants and CWD prions appears to be strong considering the low attack rate of most CWD inoculums in cattle (mule deer inoculum: 5/13, elk: 2/14) and sheep (mule deer: 1/8) (Hamir et al. 2005, Hamir et al. 2006, Greenlee et al. 2012). The high attack rate (11/12) exhibited by CWD inocula from white-tailed deer in cattle could indicate potential susceptibility via a more natural transmission route, but this would most likely require a larger infectivity dose (Hamir et al. 2007). Cattle naturally exposed to CWD PrP^{Sc} via oral challenge (mule deer brain) or cohabitation with infected captive

[6] https://www.oie.int/en/animal-health-in-the-world/oie-wahis-wild/

cervids (mule, white-tailed deer, and elk) (Miller and Wild 2004) in contaminated rangeland failed to develop prion disease during the 10 years the experiment lasted (Williams et al. 2018). Altogether, these data show that under realistic conditions, the species barrier for CWD transmission between cervids and cattle may be strong and the transmission risk low.

Another issue related to foodborne transmission to humans is whether CWD prions amplified in livestock are readily available for human consumption. Following an intracerebral CWD challenge in cattle, Haley et al. (2016) found that CWD PrP^{Sc} in infected animals were mostly restricted to the CNS and had very limited deposition in peripheral tissues, a situation resembling BSE prion distribution. Thus, removal of specific high-risk material prior to human consumption, as currently done in the cattle industry to prevent human exposure to BSE (Valleron et al. 2001), may further reduce the chance of humans being exposed to a novel CWD-related prion disease in livestock.

Further features observed among natural hosts of CWD include accumulation of PrP^{Sc} in the skeletal musculature (Angers et al. 2006), fat (Race et al. 2009), and antler velvet (Angers et al. 2009) meaning that direct human exposure to CWD PrP^{Sc} might arise either by meat consumption or by certain alternative medicine practices. Examination of available epidemiological data has not identified increases in the incidence or risk of CJD in areas where CWD has been described (Pape et al. 2006). Likewise, no causal link between CJD and hunting or consumption of game meat has been identified (Belay et al. 2001). Neuropathological cases with a clinical history suggestive of CWD transmission to humans have been shown to be caused by other protein misfolding disorders (Anderson et al. 2007). Moreover, among 81 individuals with known exposure to CWD infected deer, no CJD was diagnosed after six years follow-up (Olszowy et al. 2014). It is important, however, that these findings regarding human exposure to CWD PrP^{Sc} should be considered with caution, given the extraordinarily lengthy incubation periods (decades) that prion diseases can undergo (Scheckel and Aguzzi 2018).

The transmission potential of CWD PrP^{Sc} to humans has been experimentally investigated in nonhuman primates with contrasting results over similar observation periods (Race et al. 2009). Following intracerebral and oral challenge with CWD inoculum, squirrel monkeys (*Saimiri sciureus*) developed either clinical signs compatible with CWD infection within 33-53 months (intracerebral) or showed PrP^{Sc} deposits in the brain and/or peripheral lymphoid tissues (oral). In contrast, cynomolgus macaques (*Macaca fascicularis*) remained infection free following the same routes of challenge. Moreover, 11 to 13 years after their original challenge, the

remaining macaques continued to be disease free even when tested with the highly sensitive real time quaking-induced conversion (RT-QuIC) assay (Race et al. 2018). These results contrast with another decade-long study carried out by a group from Canada and Germany that similarly attempted to transmit CWD to macaques, in which preliminary results suggest efficient transmission (VKM 2017). The strength of these preliminary/final data have, however, not yet been scrutinized by peer-review. In the meantime, Race et al. (2018) have stated that the main disease-specific feature reported by the Canadian-German group (i.e. PrP^{Sc} staining in the spinal cord by IHC) was observed in both uninoculated and CWD-inoculated macaques in their study. Macaques are evolutionarily closer to humans than squirrel monkeys (Race et al. 2009), indicating that CWD transmissibility experiments in macaques are important to better understand the potential risk for humans. So far, the zoonotic risk of CWD appears to be very low. Nonetheless, the discrepancies between studies do not allow the possibility of CWD transmission to humans to be completely ruled out.

The BSE-vCJD pathway discussed above depicts that prion adaptation into new host species is possible in real life conditions, creating novel risks for human health. For instance, although human dietary exposure to scrapie has occurred for hundreds of years, there is no epidemiologic evidence to suggest its zoonotic transmission. However, mice models have recently estimated a possible zoonotic potential for scrapie (Cassard et al. 2014, Comoy et al. 2015). *In vitro* assays suggest that CWD PrP^{Sc} adapts more readily to new host species than BSE (Davenport et al. 2018), strengthening the unpredictable nature of prions. In light of interspecies transmission of prion diseases representing a latent risk, the take home message should be that CWD prion contact with humans should be minimized (Williams and Miller 2002).

2.2 *PRNP*: the prion protein gene

As introduced earlier, PrP^{Sc} together with PrP^{C} actively plays a role in the physiopathology of prion diseases. Nonetheless, PrP^{C} is a functional protein, encoded by the host DNA, specifically by the prion protein gene (*PRNP*). As PrP^{C} is an active player in prion diseases, variability at the *PRNP* locus has a tremendous impact on the pathology of these diseases evidenced by: (1) PrP^{C} depletion as a result of *PRNP* disruption leads to disease resistance (Büeler et al. 1993), and (2) non-synonymous substitutions in *PRNP* can change disease onset and progression. This section further describes *PRNP* organization with special focus on the coding sequence and the effects that *PRNP* variability has on various aspects of CWD transmission and pathology.

PRNP is member of the **prion gene family** together with its paralogs *PRND* (downstream prion protein-like) and *SPRN* (shadow of prion protein) (Lee et al. 1998, Makrinou et al. 2002) that also are protein-coding genes, respectively for the Doppel and Shadoo proteins (Harrison et al. 2010). Only certain structural features of the prion protein are shared with either of its paralogs, for instance, Doppel displays a conserved C-terminal domain and Shadoo a conserved N-terminal domain (Harrison et al. 2010). Further generalities on the prion gene family will be discussed before centering the attention into *PRNP* and its structure.

The gene organization is similar across all members of the prion gene family, ranging from two to three exons (Premzl and Gamulin 2007). The promoters of *PRNP* and *SPRN*, but not that of *PRND*, contain a CpG island. This promoter feature may relate to the broader expression of *PRNP*[7] and *SPRN*[8] compared to the tissue specific *PRND* (Makrinou et al. 2002, Premzl and Gamulin 2007). Yet, *PRNP* expression is the most ubiquitous of the prion gene family, and intron 1, one of the most conserved regions in *PRNP*, spans binding sites for various transcription factors essential for the proper functionality of the promoter region (Kim et al. 2008).

In the 3' terminal exon, *PRNP* encodes the complete primary sequence of PrP^C (Gabriel et al. 1992, Lee et al. 1998, Simonic et al. 2000, Premzl et al. 2005), which is preserved across vertebrates[9]. Phylogenetic analysis of the amino acid sequences encoded by *PRNP*, *SPRN* and *PRND* in vertebrates separates the primary sequence of mammalian PrP^C from that of other vertebrates (Premzl and Gamulin 2007), indicating that the most structural elements are highly conserved in mammals. While a high amino acid identity (~90%) is found among mammalians (Wopfner et al. 1999), this level is much lower when mammals are compared to other vertebrates.

Despite the amino acid sequence discrepancy between vertebrates, most structural features of PrP^C are conserved in higher vertebrates, namely mammals, birds, reptiles, amphibians and marsupials (Gabriel et al. 1992, Simonic et al. 2000, Strumbo et al. 2001, Calzolai et al. 2005, Christen et al. 2008, Christen et al. 2009). These conserved PrP^C features include: (1) a flexible N-terminal tail that consists of a signal peptide and a repeat rich region followed by (2) a conserved hydrophobic region with a palindromic sequence AGAAAAGA (Norstrom and Mastrianni 2005) and a glycine rich region (Harrison et al. 2010) (**Figure 2**) that extends to (3) a structured C-

[7] https://www.proteinatlas.org/ENSG00000171867-*PRNP*/tissue

[8] https://www.proteinatlas.org/ENSG00000203772-SPRN/tissue

[9] https://www.ncbi.nlm.nih.gov/gene/5621/ortholog/?scope=32523

terminal globular domain with three α-helices and a two-stranded β-sheet (Donne et al. 1997, Riek et al. 1997, van Rheede et al. 2003).

Figure 2 displays features from the N-terminal tail and hydrophobic region in the primary structure of PrP^C from six mammal species including mule deer as a *Cervidae* representative. The localization of main features in the C-terminal globular domain vary between species and are not displayed in **Figure 2**. Given that *Cervidae PRNP* is the topic of this thesis, focus on the features of this gene, including the globular domain of cervid PrP^C, will be further explained and illustrated in the next section (2.2.1). Before that, I will conclude with some final comments on conserved elements in the N-terminal tail and hydrophobic region of mammalian *PRNP*.

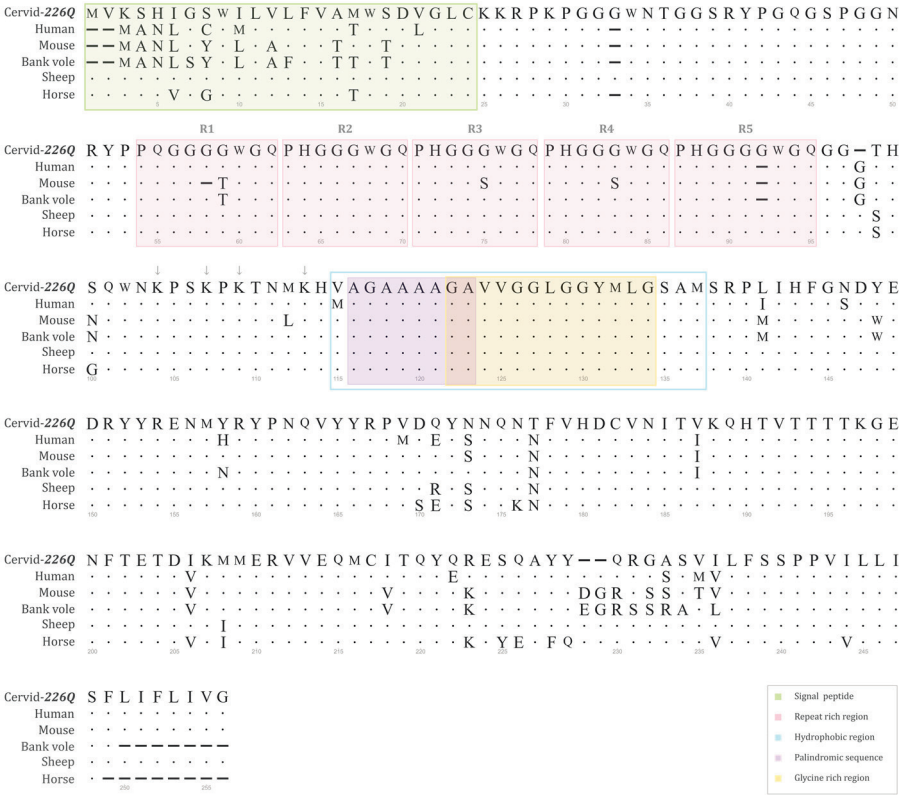

Figure 2*. Primary structure comparison of full-length cervid PrP-226Q and selected species based on multiple sequence alignment by ClustalW. Amino acid sequences were retrieved from GenBank for mule deer as cervid-226Q (O. h. hemionus, AAO91945.1), human (Homo sapiens, AAA60182.1), mouse (Mus musculus, AAA39997.1), bank vole (Myodes glareolus, AAL57231.1), sheep (Ovis aries, NP_001009481.1) and horse (Equus caballus, ABL86003.1). Amino acid numbering is based on cervid-226Q.*

In **Figure 2**, we observe that the middle region linking the flexible and globular domains has many highly conserved features. These include a cluster of four lysines (Wang et al. 2010), and a hydrophobic region enclosing the palindromic sequence AGAAAAGA (Norstrom and Mastrianni 2005) and a glycine rich region (Harrison et al. 2010). These areas in the hydrophobic region, highlighted in **Figure 2**, are common in many species (Harrison et al. 2010) and have been shown to likely represent an important modulator for PrP^{Sc} conversion (Abskharon et al. 2019).

Compared to other vertebrate classes, a distinctive feature of the mammalian N-terminal tail is conservation of the repeat region (Premzl et al. 2005). Within the Eutherian clade (i.e. placental mammals), this region has a homogeneous five repeat composition (Martin et al. 2009) of either solely octapeptides or nona- and octapeptides combined (Kim et al. 2008) (**Figure 2**). An extra octapeptide is documented among certain ruminants e.g. *Bovinae*, *Antilopinae* and *Giraffidae* and occasionally in some small mammals (Martin et al. 2009) including the squirrel monkey (Kim et al. 2008). Variability in the number of octapeptide repeats occurs within and among mammalian species and it is the main contributor for length differences in *PRNP* coding sequences (Rongyan et al. 2008). Within species, a larger number of repeats is suggested to be associated with prion disease susceptibility (Kim et al. 2008).

2.2.1 *PRNP* structure in cervids

In cervids, chromosome 13 harbors *PRNP* (Seabury et al. 2020), which was first described in mule deer by Brayton et al. (2004). The cervid *PRNP* (**Figure 3**) is 19.9kb long and consists of three exons (1: 54 bp; 2: 98bp; 3: 4083 bp) interspersed with two introns (1: 2407 bp; 2: 13252 bp). The complete open reading frame is located in exon 3 (Brayton et al. 2004). Subsequently, Seabury et al. (2007) characterized a putative promoter region in elk (2.2 kb) and found this to be similar to that in mule deer (Brayton et al. 2004). Sequence similarities between these two species also included exon 1 (identical) extending 193 bp into intron 1 (Seabury et al. 2007), probably reflecting roles for these regions in regulating expression of PrP^{C} (Haigh et al. 2007, Wright et al. 2009). The putative *PRNP* promoter in cervids includes conserved motifs among mammals including a CpG island and potential binding sites for transcription factors AP-2 and Sp1 (Seabury et al. 2007).

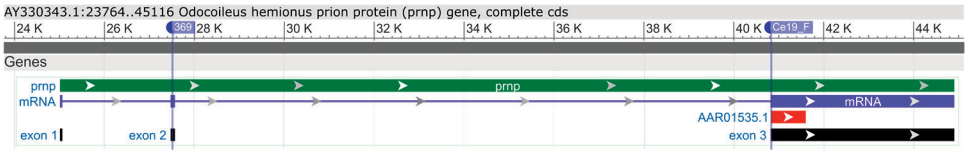

Figure 3*. PRNP in cervids spans in 19.9kb (green) and organizes into three exons (black). The coding region locates in exon 3 (red, e.g. AAR01535.1) which translates into the prion protein. Annealing sites for primers for selective amplification of either PRNP (Ce19_F) or pseudogene PRNPψ (369) paired with primer Ce778_R (not shown) are indicated in blue. Source: https://www.ncbi.nlm.nih.gov/projects/sviewer/?id=ay330343#*

In addition to *PRNP* – the functional gene, an untranslated pseudogene (*PRNPψ*) has been identified in mule deer and in white and black-tailed deer but not in elk, moose, reindeer or fallow deer (Brayton et al. 2004, O'Rourke et al. 2004). *PRNPψ* possesses the distinctive features of a processed pseudogene being flanked by direct repeats, a lack of introns and a 3' poly(A) tract. In addition to the lack of introns, alignment with the functional gene shows that *PRNPψ* is truncated by 25bp at the 5' end of exon 1, which results in *PRNPψ* being shorter than the functional gene (4223bp without poly (A) tract and direct repeats) (Brayton et al. 2004). Another hallmark differentiating *PRNPψ* from the functional gene is in the coding sequence, also found in exon 3 (O'Rourke et al. 2004). Whereas the functional gene encodes a serine (Ser) at codon 138, *PRNPψ*, constitutively encodes an asparagine (Asn). Nonetheless, the substitution Ser138Asn can be found in *PRNP* from cervid species that lack *PRNPψ*, e.g. fallow deer and reindeer/caribou (O'Rourke et al. 2004). Interestingly, Ser138Asn has been associated with reduced susceptibility to CWD (Mitchell et al. 2012). Conspecific substitutions can be found in *PRNPψ* such as Gly65Glu and Arg151Cys in mule deer (Brayton et al. 2004), and an additional octapeptide, in white-tailed deer (O'Rourke et al. 2004, Johnson et al. 2006). However, there is no evidence that *PRNPψ* presence associates with CWD susceptibility (O'Rourke et al. 2004, Johnson et al. 2006, Kelly et al. 2008).

PRNP variability, particularly non-synonymous substitutions, can profoundly influence CWD susceptibility (Brayton et al. 2004, O'Rourke et al. 2004). *PRNP* is the most important genetic component for CWD as recently reported by a genome-wide association analysis in farmed white-tailed deer (Seabury et al. 2020). Cervid *PRNP* has features that result in structural rearrangement of the tertiary structure, distinguishing cervid PrP^C from other mammals.

2.2.2 PrP^C: the prion protein, the structure in cervids

The coding sequence in cervid *PRNP* consists of 771 nucleotides that translate into 256 amino acids constituting the primary structure of cervid PrP^C. There are two predominant primary structures in *Cervidae* which are identical, with the exception of codon 226, which encodes either glutamine (Gln; Q) or glutamate (Glu; E) (Robinson et al. 2012). These **standard sequences** will be referred to as ***226Q*** and ***226E*** respectively. The nuclear magnetic resonance (NMR) structure of cervid PrP^C displays a folding pattern roughly similar to other mammalian PrP^C with a highly disordered N-terminal domain and a well-defined C-terminal domain (Gossert et al. 2005, Slapšak et al. 2019). During biosynthesis, the N- and C- terminal signal sequences are removed, and a glycosylphosphatidylinositol (GPI) anchor is added for C-terminal membrane attachment of the mature PrP^C (Linsenmeier et al. 2017).

The flexible domain in cervids (**Figure 2**) encodes the N-terminal signal peptide starting with MVKSH, common to most Eutherians except for a specific clade (*Euarchontoglires*) that includes primates and rodents (van Rheede et al. 2003). The repeat region is structured as one nonapeptide (PQGGGGWGQ) – three octapeptides (PHGGGWGQ) – one nonapeptide (PHGGGGWGQ), an arrangement also found in even-toed ungulates (i.e. pig, camel, small ruminants), and carnivores (dog, cat, mink) (Kim et al. 2008). The hydrophobic region preserves the AGAAAAGA palindrome (residues 116-123) and glycine rich region (residues 122-134), similarly observed in other mammals (van Rheede et al. 2003, Harrison et al. 2010), birds (Wopfner et al. 1999) and marsupials (Premzl et al. 2005).

The globular domain in *Cervidae* (*226Q* and *226E*) resembles that in other mammals with three α-helices and a two-stranded β-sheet (**Figure 4A**), yet this domain stands out on its tertiary structure (**Figure 5**). The loop linking β-strand 2 and the helix α2 (hereafter referred as the β2-α2 loop), which is structurally disordered in most mammals, is well defined in cervids (Gossert et al. 2005, Slapšak et al. 2019) and is accounted for by two conserved residues in the cervid loop: asparagine (Asn) 173 and threonine (Thr) 177 (Gossert et al. 2005) (**Figure 4B**). The structural order of the β2-α2 loop allows long-range effects on helix α3 (Gossert et al. 2005, Pérez et al. 2010), where residue 226 is localized. This not only differentiates the two predominant primary structures described in *Cervidae* but has also been suggested to impact the structural interactions within cervid PrP^C (Slapšak et al. 2019).

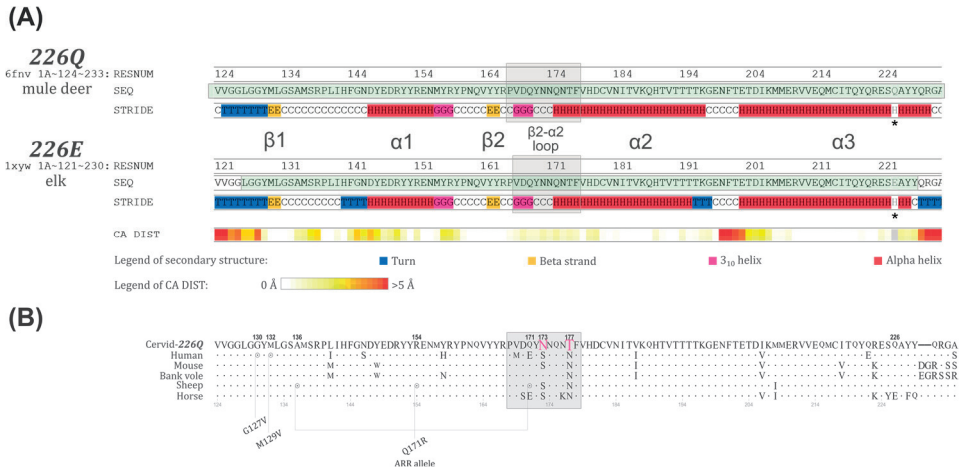

Figure 4. ***(A)*** *Secondary structure comparison between the most common cervid PrP (226Q and 226E) based on STRIDE assignment. On display, polypeptide fragments that contain the C-terminus domain (shaded green) of cervid 226Q (6FNV) and 226E (1XYW). An asterisk (*) indicates the distinctive codon 226. CA DIST depicts this spatial variability by indicating the distance per residue between aligned C-alpha, which indirectly indicates the large structural differences between these two conformers. Structure image prepared using the webserver 2StrucCompare (Drew and Janes 2019) under the STRuctural IDEntification (STRIDE) assignment (Frishman and Argos 1995).* ***(B)*** *Primary structure comparison of partial cervid PrP-226Q and selected mammal species based on multiple sequence alignment by ClustalW. On display is the region between codons 124-233 of mule deer-226Q (O. h. hemionus, AAO91945.1, 124~233) representing cervids. The matching region is also shown for human (121~230), mouse (120~231), bank vole (121~232), sheep (124~233) and horse 123~232). Amino acid comparisons, numbering and β2-α2 loop shading are based on cervid-226Q. Amino acids accounting for the structured loop in cervid PrP are colored. Variant positions modulating susceptibility to prion diseases in humans and sheep are encircled and numbered after the correspondent species numbering. Labeling in both images with Inkscape 1.0.1 (RRID:SCR_014479).*

The effect of residue 226 can be visually compared by plotting the structural arrangements of *226Q* and *226E*, publicly available as mule deer (6FNV)[10] and elk

[10] https://www.rcsb.org/structure/6FNV

(1XYW)[11] conformers. Based on NMR interactions, **Figure 4A** shows that the secondary structure is shared between mule deer (*226Q*) and elk (*226E*) PrP^{C}; although there are some structural differences. **Figure 5** compares the tertiary structure of *226Q* and *226E* where, as described by Slapšak et al. (2019), differences appear in the long-range interactions between β2-α2 loop with α3-helix, between the three α-helices and in the electrostatic surface potential. The structural rearrangements distinguishing the two predominant cervid PrP^{C} conformers may be relevant in PrP^{C} and PrP^{Sc} interaction (Slapšak et al. 2019) and could explain the distinctive CWD prion replication, pathogenesis, and biochemical properties solely dictated by residue 226 in cervid PrP^{C} (Bian et al. 2019).

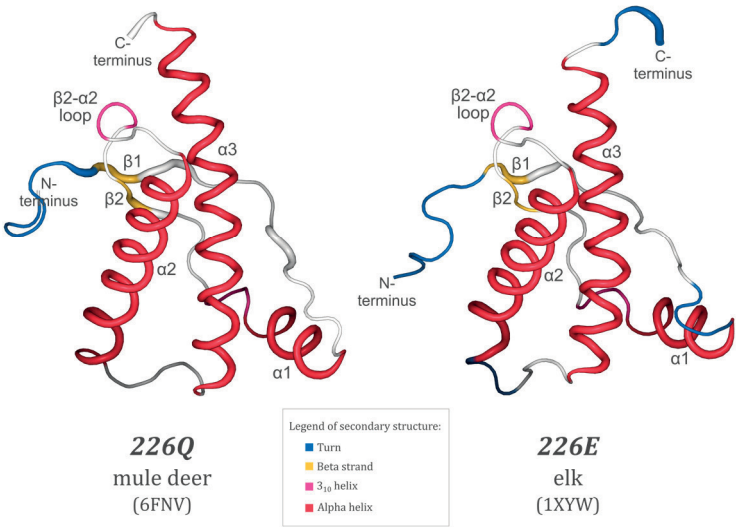

***Figure 5**. Tertiary structure comparison of the most common cervid PrP (226Q and 226E) based on their NMR structure. On display is the C-terminus domain of cervid 226Q (6FNV) and 226E (1XYW). The end of the α3 helix is longer in 226Q resulting in long range interactions of this area with the β2-α2 loop in 226Q compared to 226E (Slapšak et al. 2019). Structure images prepared using the webserver 2StrucCompare (Drew and Janes 2019), colored under the STRIDE assignment (Frishman and Argos 1995) and labelled with Inkscape 1.0.1 (RRID:SCR_014479).*

[11] https://www.rcsb.org/structure/1XYW

2.2.3 Describing *PRNP* variation

Cervids are diploid and thus inherit a pair of *PRNP* genes from the parents. Sequence variations have been commonly termed polymorphisms or mutations, but I will instead use the term "variant" following the Human Genome Variation Society (HGVS) recommendations to avoid the imprecise meaning and negative connotation derived from using the two former terms across disciplines (den Dunnen et al. 2016). Thus, the term single nucleotide variant (SNV) replaces single nucleotide polymorphism (SNP).

The position numbering is based on the first nucleotide/codon of *PRNP* coding sequence for descriptions at the nucleotide/protein level. Substitutions at the nucleotide level are discussed only when strictly necessary and by using the HGVS standard e.g. 674C>A (i.e. replacement of guanine by cytosine at nucleotide 674). *PRNP* variability description preferably centers at the protein level, using the IUPAC-IUB amino acid code as follows:

- The three-letter symbol e.g. Ala (for alanine) will preferably describe substitutions as in the HGVS guidelines e.g. Ser225Tyr (i.e replacement of serine by tyrosine at codon 225).
- The one-letter symbol will describe long sequences e.g. AAA (for three alanines) and the names of the *PRNP* alleles.

In this thesis, *PRNP* alleles are described at the protein level, italicized, and named by listing:

- non-synonymous substitutions e.g. *225Y*
- an underscore (_)
- a **standard sequence** (i.e. either ***226Q*** or ***226E***, see next section) on which the substitution occurs

e.g. *225Y_226Q*

If necessary, a dot (.) further separates linked substitutions e.g. *98A.168S_226Q*. The *PRNP* genotype is the combination of *PRNP* alleles that an animal inherited from its parents and when listed they are separated by a slash (/) e.g. *226Q/226Q*.

2.3 *PRNP* variation in cervids

As introduced earlier, variability in *PRNP* coding sequence determines PrP^C primary structure and such variation can associate with susceptibility to prion diseases as observed in CWD (O'Rourke et al. 2004). Many studies have therefore targeted this *PRNP* region. From now and unless otherwise indicated, *PRNP* variation will

exclusively refer to *PRNP* coding sequence when translated into its amino acid sequence (protein level). These considerations aim to simplify comparisons within the *Cervidae* family.

***Table 2**. Cervid PRNP alleles (protein level), reference DNA and protein sequences. Rare alleles are not included. The table includes species in which naturally occurring CWD has been registered in North America or are found in Norway. The asterisk (*) indicates that the reference sequence is derived from a heterozygous animal.*

Species	*PRNP* allele (protein level)	Reference sequence (DNA)	Reference sequence (protein)
Telemetacarpalian			
Mule deer	*226Q*	AY228473.1	AAO91945.1
	20G_226Q	MN389810.1	QKI86691.1
	225F_226Q	MN389800.1	QKI86681.1
White-tailed deer	*226Q*	MN390079.1	QKI86960.1
	95H_226Q	AY275711.1	AAP33274.1
	96S_226Q	AF156184.1	AAF80283.1
	116G_226Q	AY275712.1	AAP33275.1
	226K	MN390356.1	QKI87237.1
Reindeer/caribou	*226Q*	DQ154293.1	AAZ81474.1
	225Y_226Q	JQ290076.1	AFF27616.1
	deletion_226Q	MN784958.1	QHG11701.1
	176D_226Q	JQ290075.1	AFF27615.1
	2M.129S.169M_226Q	DQ154295.1	AAZ81476.1
	* *207M_226Q*	MW557843.1	QRG45871.1
	129S_226Q	DQ154294.1	AAZ81475.1
	138N_226Q	DQ154292.1	AAZ81473.1
Moose	*226Q*	MH230115.1	AZB50215.1
	109Q_226Q	JQ290077.1	AFF27617.1
	209I_226Q	MH230114.1	AZB50214.1
Roe deer	*226Q*	MK103016.1	QAU19526.1
Plesiometacarpalian			
Elk	*226E*	AF016227.2	AAC12860.2
	132L_226E	AF016228.2	AAC12861.2
Red deer	*226Q*	KT845862.1	AMP43861.1
	226E	KT845864.1	AMP43863.1
	98A_226Q	KT845863.1	AMP43862.1
	98A.168S_226Q	EU032286.1	ABS87880.1
Fallow deer	*138N_226E*	EF139175.1	ABL75511.1

The translated amino acid sequence among cervid species is well conserved with the most common *PRNP* variants encoding either *226Q* or *226E* (**Table 2**). This pattern

was described by Robinson et al. (2012) by compiling reported *PRNP* amino acid sequences from ten *Cervidae* species. Although numerous *PRNP* sequences were identified, either *226Q* or *226E* dominated in nine of the studied species. An updated and curated summary of the most reported *PRNP* variants in cervid species with either natural or experimental CWD infection is shown in **Figure 6**. These species include mule deer, white-tailed deer, caribou/reindeer, moose, red deer, elk, and fallow deer as in **Table 2**.

In addition to the variant positions depicted in **Figure 6**, other non-synonymous substitutions have been reported among the species listed in **Table 2** e.g. Gly37Val, Gly96Arg, Ser100Asn, Asn103Ile, Ala123Thr, Gln226Arg and Gln230Leu in white-tailed deer; Tyr153Phe and Pro242Leu in caribou; Thr36Asn and Ser100Arg in moose; Lys25Arg and Thr191Ala in elk; Gly59Ser and Met208Ile in red deer (Kaluz et al. 1997, Perucchini et al. 2008, Peletto et al. 2009, Wilson et al. 2009, Wik et al. 2012, Brandt et al. 2018, Miller and Walter 2019, Arifin et al. 2020, Cullingham et al. 2020, Ishida et al. 2020). These substitutions have been identified in a few animals, occasionally as private alleles in heterozygous animals. These rather rare alleles and genotypes are probably not important for CWD dynamics at the population level and will not be further discussed.

In addition to variation in the *PRNP* coding sequence, a SNV in the promoter of *PRNP* in white-tailed deer has recently been associated with CWD susceptibility (Seabury et al. 2020). The variation in the cervid *PRNP* promoter is different from that in livestock ruminants (Heaton et al. 2003) and includes both SNVs and deletions (Heaton et al. 2003, Zink et al. 2020). Whether these changes in the promoter affect CWD susceptibility is currently not known and will thus not be discussed further in this thesis.

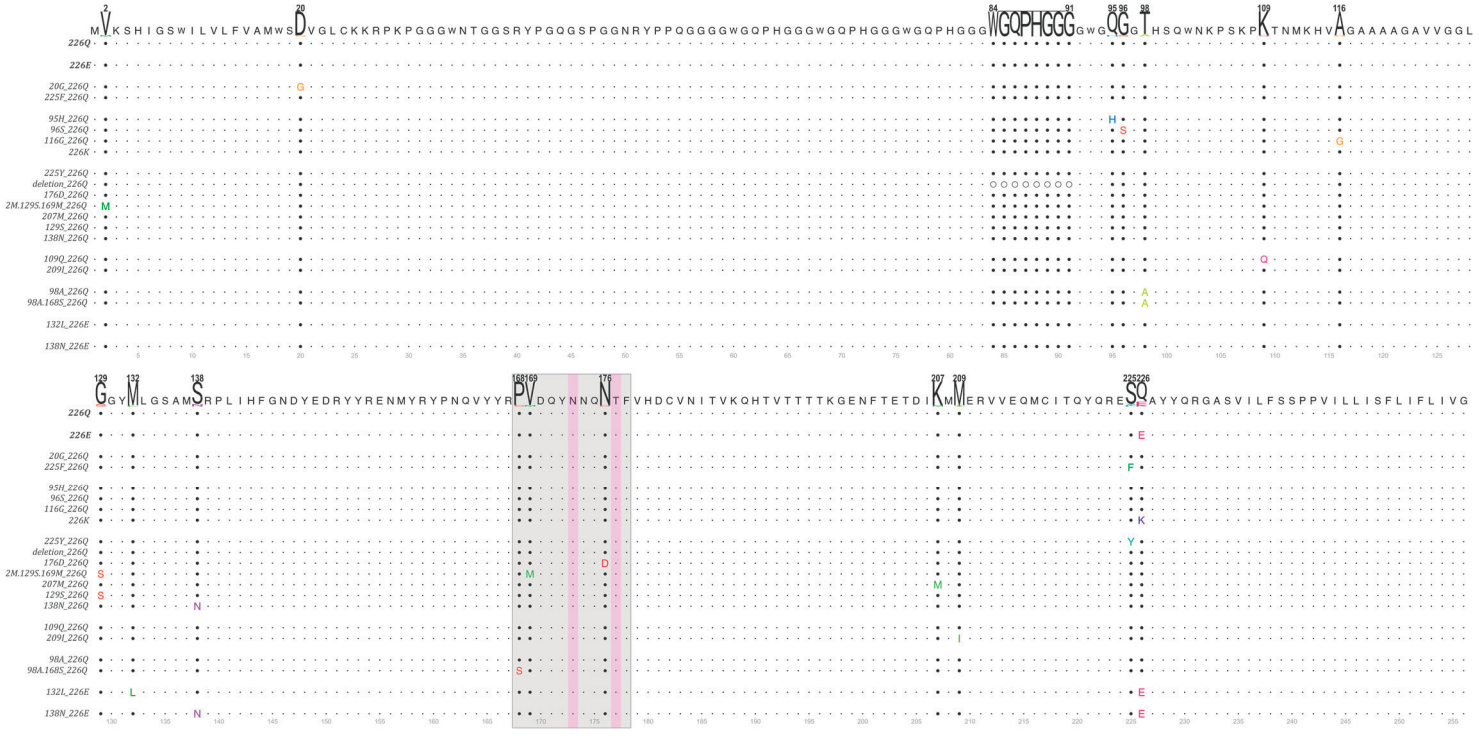

Figure 6*. Alignment of common PRNP alleles (protein level) across cervid species susceptible to CWD under natural or experimental conditions. On top, a sequence logo summarizes the pattern of variability at each codon. Variant positions are numbered in relation to reference sequence 226Q. Non-synonymous substitutions are colored. 226Q and 226E are found in more than one species in Cervidae. Other PRNP alleles are commonly conspecific. Characteristic amino acids in cervid PrP^C associated with the β2-α2 loop (shaded gray based on 226Q) are shaded fuchsia. See* ***Figure 7*** *for allocation by species and* ***Table 2*** *for detailed information on the GenBank identifiers.*

2.3.1 *PRNP* variation in cervids and its relationship with CWD

As previously mentioned, (section 2.1.2.1), investigations of CWD occurrence in mule deer, white-tailed and elk have provided valuable epidemiological information, linking *PRNP* variation to CWD susceptibility. Most of these CWD cases have an overrepresentation of either ***226Q*** -mule deer (Brayton et al. 2004, Wilson et al. 2009), white-tailed deer (Johnson et al. 2006)- or ***226E*** -elk (O'Rourke et al. 1999, Spraker et al. 2004). These *PRNP* amino acid sequences among cervids follow the phylogenetic organization of the *Cervidae* family into two lineages (**Figure 7**): *telemetacarpalian* and *plesiometacarpalian*. The main phylogenetic relationships are also reflected in the coding sequence of *PRNP* (Rongyan et al. 2008).

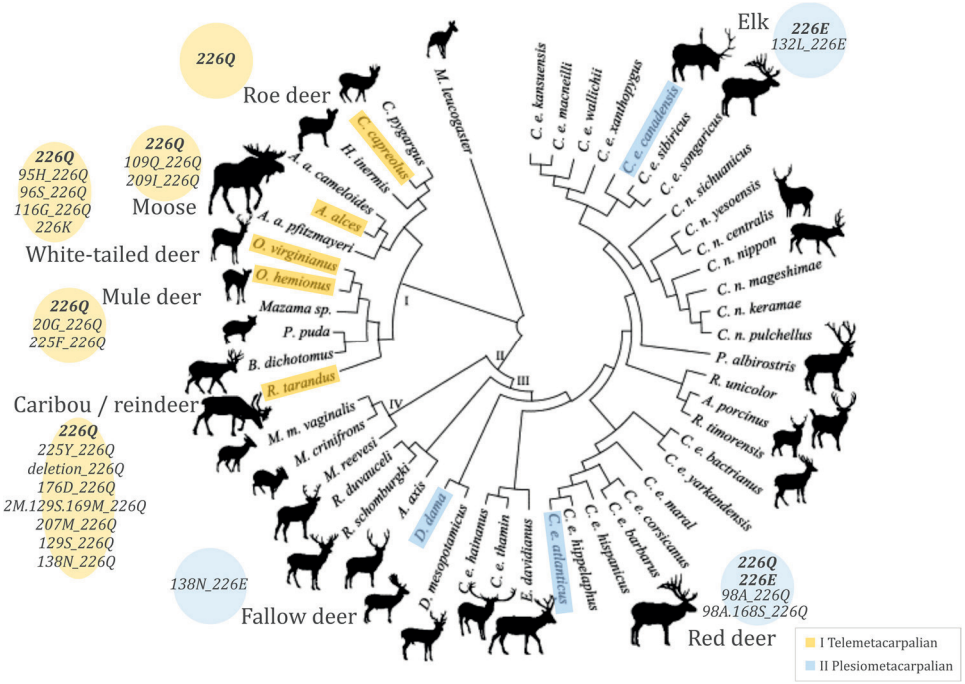

Figure 7*. Consensus tree showing the intrafamilial relationships within the family Cervidae -based on cyt b gene sequences from 49 cervid taxa- and commonly observed PRNP alleles (protein level) in species as listed in* ***Table 2****.* ***Figure 7*** *is a derivative of "Fig. 1." by Pitra et al. (2004) (https://doi.org/10.1016/j.ympev.2004.07.013), used under Elsevier user license.*

In the phylogenetic tree by Robinson et al. (2012), *226Q* was primarily found among members of the *telemetacarpalian* lineage (referred as subfamily *Capreolinae*)

whereas *226E* was only found in members of the *plesiometacarpalian* lineage (referred as subfamily *Cervinae*). Yet some species constitutively encode a different *PRNP* allele e.g. fallow deer encoding Ser138Asn against the *226E* background (*138N_226E*) (Rhyan et al. 2011, Wik et al. 2012). Interestingly, *138N_226E* appears to be associated with lower susceptibility to CWD compared with *226Q* and *226E*. CWD has only been observed after intracerebral inoculation in fallow deer (Hamir et al. 2011), despite exposure to either contaminated pastures or cohabitation with infected white-tailed deer (Rhyan et al. 2011). Additional amino acid substitutions on the background of *226Q* and *226E* and the resulting amino acid sequences can be referred as "additional *PRNP* variants" distinct from *226Q* and *226E*. A summarized distribution of *226Q*, *226E* and additional *PRNP* variants relative to *Cervidae* phylogeny is shown in **Figure 7**.

These additional *PRNP* variants raise PrP^{C} conformers different from *226Q* and *226E*, which appear to be mostly conspecific (**Figure 7**). All these *PRNP* variants in *Cervidae*, constitutively encode Asn173 and Thr177 (**Figure 6**), which are important determinants of the outstandingly well-defined β2-α2 loop in cervid PrP^{C}. Therefore, it is predicted that this feature is conserved in the *PRNP* variants described in **Table 2**. The β2-α2 loop and the C-terminal segment of α3 helix in the cervid PrP^{C} have been proposed to constitute a PrP^{Sc} conversion epitope. The plasticity of this epitope may mediate how readily accessible it becomes on the protein surface (Pérez et al. 2010). The molecular localization and/or the physicochemical dissimilarity encompassed by substitutions occurring in the *226Q* and *226E* background may structurally impact the plasticity of the so -called conversion epitope in the globular domain, or affect conserved key areas in the middle region and flexible domain of PrP^{C}. Whereas functional studies of *PRNP* would provide new insights into the biological significance of the genetic variants, epidemiological data on the distribution of the different *PRNP* variants can provide clues on their impact on CWD susceptibility. The correlation between *PRNP* variants and CWD infection status frequently derives from observational studies following natural outbreaks. Experimental studies in cervids and animal models (e.g. mice and bank vole) have added valuable information to the current knowledge of CWD dynamics. An overview on the three most commonly CWD affected species and their *PRNP* variants will be presented in this section.

2.3.1.1 Mule deer

Among wild mule deer diagnosed with CWD, most animals (~70%) are found to be homozygous for *226Q* (Brayton et al. 2004, Wilson et al. 2009). Other *PRNP* variants, such as *20G_226Q* and *225F_226Q* are also found but in combination with *226Q* as

heterozygous (Brayton et al. 2004, Wilson et al. 2009) and rarely as homozygous (Brayton et al. 2004, Jewell et al. 2005).

The odds of being CWD positive, which indicates susceptibility to CWD, is reduced in animals carrying a single copy of allele *225F_226Q* (i.e. 225SF) compared with those carrying none (Jewell et al. 2005, DeVivo et al. 2017). Similarly, the deposition of PrP^{Sc} in the lymph nodes and in the CNS tissue is much slower in animals heterozygous for *225F_226Q* than in animals without *225F_226Q* (Fox et al. 2006). Mule deer homozygous for allele *225F_226Q* exposed to a CWD contaminated paddock had a delayed disease onset and more subtle clinical signs than the other genotype (i.e. 225SS, most probably *226Q*/*226Q*) and this prolonged clinical course resembled that of CWD cases homozygote for allele *225F_226Q* (Wolfe et al. 2014). Thus, allele *225F_226Q* is considered to award reduced susceptibility to CWD.

2.3.1.2 White-tailed deer

White-tailed deer is an interesting species given the high variability of their *PRNP* coding sequence. At least 40 *PRNP* coding sequences have been assigned a unique identifier i.e. haplotypes A-Z, *PRNP*-Odvi27 to *PRNP*-Odvi38, AR1, AR2 (Brandt et al. 2015, Brandt et al. 2018, Chafin et al. 2020, Ishida et al. 2020) from which 16 are predicted to encode *226Q* (haplotypes A, B, D, E, G, H, J, O, R, T, *PRNP*-Odvi31[12] to *PRNP*-Odvi33, *PRNP*-Odvi36, *PRNP*-Odvi38, AR1) and 11 are predicted to encode *96S_226Q* (haplotypes C, I, P, V, W, X, Z, *PRNP*-Odvi27, *PRNP*-Odvi28, *PRNP*-Odvi34[13], AR2). Not surprisingly, *226Q* and *96S_226Q* are the major *PRNP* variants among wild and farmed white-tailed deer, although other variants are also observed (Haley et al. 2016, Brandt et al. 2018, Chafin et al. 2020, Ishida et al. 2020).

Despite this great variety, the most common allele among CWD infected white-tailed deer is *226Q*, contrasting with a low proportion of *96S_226Q*. This pattern becomes more evident in free-ranging animals (Johnson et al. 2006, Kelly et al. 2008, Brandt et al. 2015, Haley et al. 2016, Brandt et al. 2018, Chafin et al. 2020, Ishida et al. 2020). Other *PRNP* variants e.g. *95H_226Q* (haplotypes CCTCGGCCC, F, allele HGAQ, *PRNP*-Odvi35, *PRNP*-Odvi37) (Kelly et al. 2008, Brandt et al. 2015, Haley et al. 2016, Brandt et al. 2018, Ishida et al. 2020) and *226K* (haplotype K, allele QGAK, Q226K) (Johnson et al. 2006, Keane et al. 2008, Haley et al. 2016, Chafin et al. 2020, Ishida et al. 2020) are rarely reported (ca. 1%). So, despite a range of genotypes in white-tailed deer,

[12] Like haplotype AR4 reported by Chafin, et al. 2020

[13] Like haplotype AR3 reported by Chafin, et al. 2020

CWD cases mainly involve *226Q*/*226Q* and the frequencies of these genotypes are commonly higher in free-ranging animals (ca. 80%) (Johnson et al. 2006, Brandt et al. 2015, Ishida et al. 2020) than in farmed white-tailed deer (ca. 50-70%) (Keane et al. 2008, Haley et al. 2016, Haley et al. 2019).

Evidence points to *226Q*/*226Q* increasing the risk of CWD infection compared to genotype *226Q*/*96S_226Q* (Keane et al. 2008), whereas the risk of CWD infection is significantly reduced for most *PRNP* genotypes carrying at least one copy of *95H_226Q*, *96S_226Q*, *116G_226Q* and *226K*, except for *226Q*/*226K* (referred as 96G/226K) compared to *226Q*/*226Q* (referred as 96GG genotype) (Haley et al. 2019). On the other hand, haplotype C i.e. *96S_226Q* (Brandt et al. 2015, Brandt et al. 2018, Chafin et al. 2020) and haplotype F i.e. *95H_226Q* (Brandt et al. 2018) are significantly associated with a reduced likelihood of being infected with CWD, a pattern significantly more pronounced in animals with genotypes solely based on the combination of these two alleles (Ishida et al. 2020). Moreover, SNVs in the coding sequence of *PRNP* at positions 285A>C (Gln95His) and 286G>A (Gly96Ser) are found to be "protective" against CWD, which respectively act as markers for *95H_226Q* and *96S_226Q* (Kelly et al. 2008). The association between carriers of either *95H_226Q* or *96S_226Q* and a reduced CWD risk is supported by how these alleles affect disease progression.

The presence of *95H_226Q* or *96S_226Q* significantly associates with extended incubation periods (Johnson et al. 2011, Hoover et al. 2017). This can be related to *PRNP* variants either limiting or delaying early lymphoid amplification of the CWD prion (Johnson et al. 2006, Hoover et al. 2017, Otero et al. 2019), which appears to precede neuroinvasion and CWD development (Hoover et al. 2017). Moreover, data from depopulated farms in North America show that CWD affected animals carrying *95H_226Q*, *96S_226Q* and *116G_226Q* were identified at significantly earlier stages of disease than those only carrying *226Q* at the time of depopulation (Haley et al. 2019). In the same study, a similar trend was observed with *226K*, but this was not statistically significant. Altogether, this data indicates *95H_226Q* and *96S_226Q* to award reduced susceptibility to CWD with likely similar trends for *116G_226Q* and *226K*.

2.3.1.3 Elk

Variability in the *PRNP* coding sequence of elk is limited, with most animals encoding *226E*. *226Q* has not been reported in this species. The other *PRNP* variant *132L_226E* (see haplotype Elk*PRNP*-3, haplotypes 2 and 7) (Seabury et al. 2007, White et al.

2010) occurs at lower frequencies, which are slightly more marked in free-ranging compared to farmed animals (O'Rourke et al. 1999, White et al. 2010).
Among CWD-affected elk, *226E* is clearly over-represented and animals homozygous for *226E* often signify more than 70%, whereas the rest carry a copy of *132L_226E* (O'Rourke et al. 1999, Spraker et al. 2004). On the other hand, elk naturally infected with CWD harboring two copies of *132L_226E* are seldom found (Spraker et al. 2004, Hamir et al. 2006, Haley et al. 2020). Nonetheless, a study in wild elk showed either combination of *226E* and *132L_226E* awarding equivalent susceptibility to CWD (Perucchini et al. 2008).
The low frequency of *132L_226E* could explain these observations. Nonetheless, Hamir et al. (2006) showed orally CWD challenged elk to differ in their time to disease onset based on their *PRNP* genotype. The CWD incubation interval was the shortest in elk homozygous for *226E* (i.e. 132MM), longer in those carrying one *132L_226E* (Hamir et al. 2006), and the longest in elk homozygous for *132L_226E*, almost three times longer than observed in elk *226E*/*226E* (O'Rourke et al. 2007). Natural cases identified in elk farms show animals carrying one *132L_226E* (i.e. 132ML) are found at an earlier disease stage than those *226E*/*226E* (Haley et al. 2016) and that the *PRNP* genotype significantly associates with the likelihood to be found CWD positive. The CWD risk among *226E*/*226E* animals is respectively two and three times that of animals heterozygous and homozygous for *132L_226E* (Haley et al. 2020). The reduced susceptibility attributed to *132L_226E* finds support in a homologous substitution in human *PRNP* where Met129Val (**Figure 4B**) associates with a reduced risk to prion diseases such as Kuru (Lee et al. 2001) and vCJD (Wadsworth et al. 2004). This suggests that changes at this position in PrP^C are biologically relevant in prion pathogenesis. Therefore, *132L_226E* is commonly regarded as awarding reduced susceptibility to CWD.

2.4 Norwegian cervids

Norway has four prevalent wild cervid species: reindeer, red deer, moose, and roe deer. In addition, farmed red deer (also a few farmed fallow deer) and semi-domestic reindeer are found. At present, the Norwegian cervid population (winter size) is roughly estimated to consist of ~25 000 wild reindeer[14], ~250 000 semi-domestic

[14] https://www.villrein.no/the-wild-reindeer-areas-in-norway

reindeer[15], ~157 000 ± 30% red deer, ~94 000 ± 30% moose and ~146 000 ± 30% roe deer (personal communication, Erling Solberg based on Speed et al. (2019) data). These numbers fluctuate between years; however, the overall moose and red deer populations have experienced a steady increase over the last 40 years (Speed et al. 2019).

Among the Norwegian cervids, reindeer ranks as the most social species, whereas, red deer form smaller social groups and moose and roe deer are more solitary (VKM 2017). There are also interesting differences concerning their population history and dynamics, suggesting that distinct colonization and historical events have influenced how Norwegian cervids are distributed and genetically structured.

2.4.1 Distribution and genetic structure

Norwegian cervids vary in their usage of land. Red deer, moose, and roe deer mostly associate with forests while reindeer are found in alpine areas (Speed et al. 2019). Reindeer include semi-domestic and wild populations. Semi-domestic reindeer clearly dominate in the north with some herds in Central Norway, whereas wild reindeer are predominantly restricted to the central part of southern Norway and distributed between 24 wild reindeer management areas (**Figure 8**). The distribution of red deer, moose and roe deer as reflected by hunting statistics (VKM 2017), a common proxy of wildlife population density, shows that moose are more widely distributed than red deer and roe deer (**Figure 9**). Across Norway, the moose density is less variable than that of red deer, which is more abundant in Western and Central Norway. Roe deer are more frequent in Eastern and Southern Norway as well as in certain areas on the west coast. Red deer farms overlap the wild red deer distribution; however, these farms have a more restricted range with most located in South-Eastern Norway (**Figure 9**). A small percentage of the red deer farms also breed fallow deer (personal communication, Lars Almo, leader of Norwegian Deer Breeding Association) which is an introduced species found only in captivity.

[15] https://www.regjeringen.no/no/tema/mat-fiske-og-landbruk/reindrift/reindrift/id2339774/

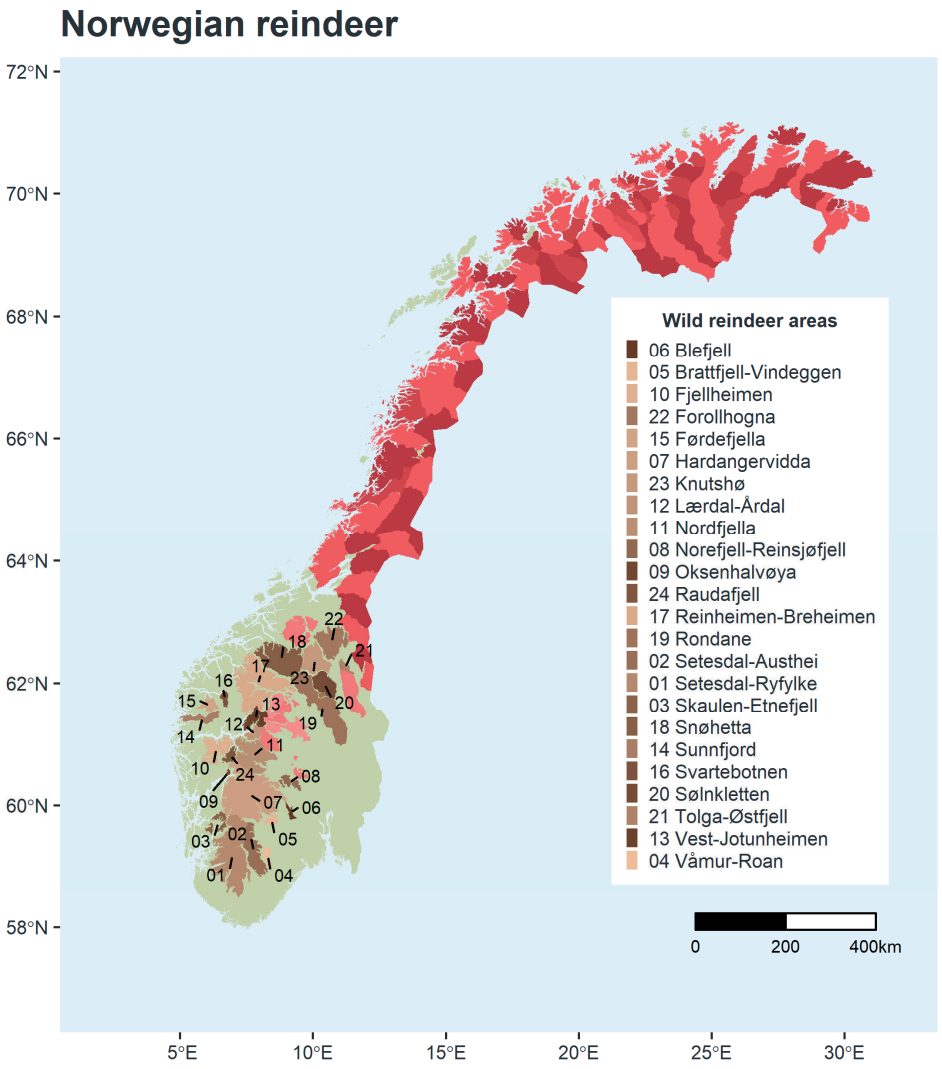

***Figure 8**. Distribution of Norwegian reindeer with 24 wild reindeer management areas (brown palette with numbers) found in the south, and semi-domestic reindeer districts (red palette) predominant in the north. Data sourced from: the Norwegian Environment agency and the Norwegian Agriculture Agency.*

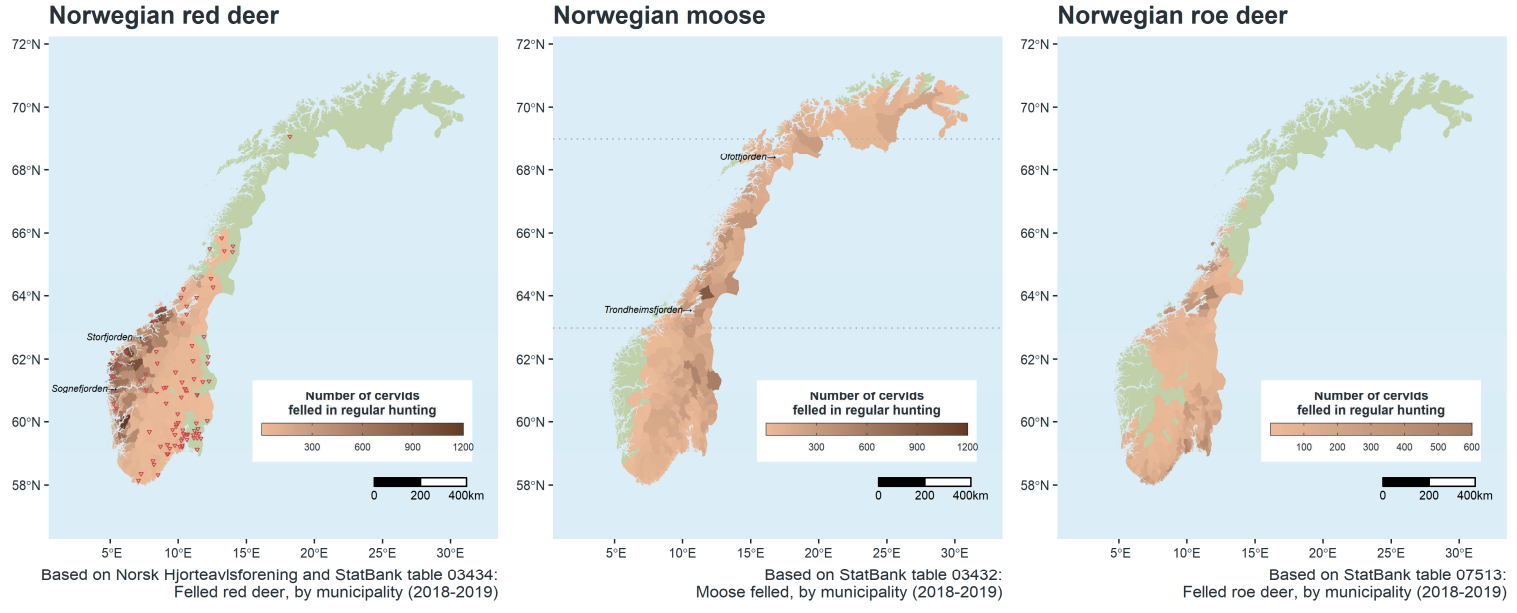

***Figure 9**. Distribution of hunted Norwegian red deer, moose, and roe deer and red deer farms. Numbers per municipality obtained for the regular hunting season 2018-2019 correspond to bar scale (brown palette) and farms location to inverted triangle (red). Data on hunting statistics sourced from Statistics Norway (https://www.ssb.no/en) and on red deer farms from Norsk Hjorteavlsforening (NHF, Norwegian Deer Breeding Association) (https://norskhjorteavlsforening.no/kart/).*

The genetic structure of Norwegian cervid populations has been influenced by their ongoing demographic history, as determined by neutral genetic markers. Wild and semi-domestic reindeer are regarded as populations of different origin (Røed et al. 2008, Røed et al. 2018). Furthermore, contemporary wild reindeer subpopulations show variable levels of introgression from semi-domestic reindeer, ranging from very low in subpopulations with mainly wild ancestry (coded 18, 19, 20, and 23 **Figure 8**), to high in subpopulations with mixed ancestry (coded 01, 02, 05, 06, 07, 11, 12 in **Figure 8**) and populations of mainly semi-domestic origin (coded 03, 04, 08, 10, 14, 15, 16, 17, 22 in **Figure 8**). The gene flow between wild reindeer management areas is generally low (Kvie et al. 2016).

Despite its continuous distribution, the genetic variation in Norwegian moose is divided by latitude into two subpopulations likely originating from two separate ancient populations in the north and south of Fennoscandia, with an area of admixture between 63°N and 69°N (Haanes et al. 2011) (**Figure 9**). Similarly, Norwegian red deer separate into two main subpopulations found north and south of Sognefjorden on the west coast with further separation into five genetic clusters associated with the drastic population reduction between the mid-18th and early 20th century. Subsequent recolonization, influenced by the steep coastline and massive alpine mountains, has contributed to the genetic structure seen today (Haanes et al. 2010). All farmed red deer originate from wild animals captured within the country (Benestad et al. 2016). Norwegian roe deer experienced a profound population decline. It is estimated that the contemporary roe deer originate from less than 100 animals in southernmost Sweden (Andersen et al. 2004) and is referred to as a cervid population of very low genetic variability (Wiehler and Tiedemann 1998).

Although Norwegian cervids are sympatric species, their demographic history has distinctively shaped their genetic structure. Consequently, the amount of genetic variability differs between species. Reindeer appear to have a genetic variability slightly higher than moose but significantly higher than red deer and roe deer (Røed 1998). Compared to the diversity found in conspecific populations across Europe, Scandinavian cervids are genetically separate (Wiehler and Tiedemann 1998, Niedziałkowska et al. 2014, Zachos et al. 2016). As in reindeer (Flagstad and Røed 2003, Yannic et al. 2014) and moose (Niedziałkowska et al. 2014), European cervid populations are generally genetically distinguishable from North American lineages. Therefore, it is expected that the differences in origin and genetic diversity may impact on the occurrence *PRNP* variants.

2.5 CWD in Europe, 2016 to 2020*

** Range based on date of animal-death not on date of diagnosis*

Norway gained the attention of the CWD research community when Benestad et al. (2016) reported the very first case of naturally occurring CWD not only in Europe, but also in reindeer worldwide. This affected animal (R-1, **Table 3**) belonged to a herd of ca. 400 wild reindeer in the Nordfjella management area (**Figure 8**, **Figure 10A**) approached by helicopter for radio-collaring purposes in March 2016 (**Figure 10B**). The animal was 3-4 years old, unable to follow the herd, was found recumbent and died shortly after. Laboratory tests including enzyme-linked immunosorbent assay (ELISA) and Western Blot (WB) identified the presence of PrP^{Sc} in brain tissues i.e. *medulla oblongata*. The findings were consistent with the standard-3 band WB pattern observed in a positive control CWD sample from North America. Likewise, the PrP^{Sc} distribution in the *medulla oblongata* and lymph nodes, as determined by IHC (Benestad et al. 2016) was consistent with that in reindeer with experimentally induced CWD (Mitchell et al. 2012).

In May 2016, ca. 300 kilometers north of Nordfjella in the municipality of Selbu, a 13 year-old moose (E-1) displaying abnormal behavior, and another 14 year-old moose (E-2) found dead in a river, were diagnosed with CWD (Pirisinu et al. 2018). In October 2017, ca. 150 kilometers north and west of Nordfjella and Selbu, a 16 year-old red deer (H-1) was diagnosed with CWD. This animal was grazing alone before being shot during regular hunting (Vikøren et al. 2019). In contrast to the IHC distribution of PrP^{Sc} in Norwegian reindeer, the brain but not the lymph nodes were positive for PrP^{Sc} in moose (E-1 to E-3) (Pirisinu et al. 2018) and red deer (Vikøren et al. 2019). By using a variety of antibodies in the WB method, it was shown that the PrP^{Sc} in Norwegian moose were different from BSE and scrapie PrP^{Sc}, as well as various North American CWD isolates, also including a Canadian moose isolate. Furthermore, the Norwegian moose isolates were distinguishable from those of Norwegian reindeer (Pirisinu et al. 2018). These differences in laboratory findings, the age bracket and the involvement of lymphoid tissues in Norwegian moose were compatible with spontaneous prion disease and the novel phenotype was denominated Nor-16CWD (Pirisinu et al. 2018).

***Table 3**. CWD cases reported in Europe (2016-2020) since the initial report in Norway. Age in categories are yearling (one year old) adult (older than one and up to eight years old), otherwise presented as years old. Data sourced from reporting authorities: Norwegian Veterinary Institute, Swedish National Veterinary Institute, and Finnish Food Authority.*

Species	Identifier	Geographic region	Sex	Age
CWD				
Reindeer	R-1	Nordfjella 1, Norway	Female	Adult
	R-2	Nordfjella 1, Norway	Male	Adult
	R-3	Nordfjella 1, Norway	Female	Adult
	R-4	Nordfjella 1, Norway	Male	Adult
	R-5	Nordfjella 1, Norway	Female	Adult
	R-6	Nordfjella 1, Norway	Male	Adult
	R-7	Nordfjella 1, Norway	Male	Adult
	R-8	Nordfjella 1, Norway	Male	Adult
	R-9	Nordfjella 1, Norway	Male	Adult
	R-10	Nordfjella 1, Norway	Male	Adult
	R-11	Nordfjella 1, Norway	Female	Adult
	R-12	Nordfjella 1, Norway	Female	Adult
	R-13	Nordfjella 1, Norway	Male	Yearling
	R-14	Nordfjella 1, Norway	Male	Adult
	R-15	Nordfjella 1, Norway	Male	Adult
	R-16	Nordfjella 1, Norway	Male	Adult
	R-17	Nordfjella 1, Norway	Male	Adult
	R-18	Nordfjella 1, Norway	Female	Adult
	R-19	Nordfjella 1, Norway	Male	Adult
	R-20	Hardangervidda, Norway	Male	Adult
Atypical CWD				
Moose	E-1	Selbu, Norway	Female	13
	E-2	Selbu, Norway	Female	14
	E-3	Lierne, Norway	Female	13
	E-4	Flesberg, Norway	Female	15
	E-5	Selbu, Norway	Female	20
	E-6	Sigdal, Norway	Female	12
	E-7	Steinkjer, Norway	Female	17
	E-8	Bamble, Norway	Male	13
	E-F:1	Kuhmo, Finland	Female	15
	E-F:2	Laukaa, Finland	Female	18
	E-S:1, VLT 541/19	Norrbotten, Sweden	Female	16
	E-S:2, VLT 876/19	Norrbotten, Sweden	Female	16
	E-S:3, CWD-0462	Norrbotten, Sweden	Female	10
	E-S:4, älg	Västerbotten, Sweden	Female	14

Species	Identifier	Geographic region	Sex	Age
Red deer	H- 1	Gjemnes, Norway	Female	16

The Norwegian Scientific Committee for Food Safety (*Vitenskapskomiteen for mattrygghet*, VKM), established a group of experts in March 2016 (Mysterud and Rolandsen 2018) to express a scientific opinion on CWD in Norway. The VKM experts initially recommended a massive surveillance of Norwegian cervids (VKM 2016). The authorities observed by January 2017 (**Figure 10B**) that all further reindeer CWD cases were limited to Nordfjella zone 1 (**Table 3**) (VKM 2017). The low connectivity between wild reindeer management areas in Norway (**Figure 8**) offered the possibility to consider the reindeer of Nordfjella zone 1 as a containable population suitable for CWD eradication. The expert group suggested an immediate strategy of depopulation, fallowing and restocking of Nordfjella zone 1 (VKM 2017). The official decision to cull the wild reindeer in Nordfjella zone 1 was reached on May 2017 (Grendstad (e.f.) and Ormsettrø 2017) with the aim of eradication of CWD. In parallel, the Norwegian surveillance program for CWD, ongoing since 2002-2003, intensified its efforts by testing of wild cervids culled during regular hunting (Våge et al. 2020). Since May 2017, further cases have been identified in reindeer (R-4 to R-18) and moose (E-3 to E-8) in Norway in addition to moose in Finland[16] (E-F:1, E-F:2) and Sweden (E-S:1 to E-S:4) (**Table 3**, **Figure 10**).

In addition to the previously mentioned differences between reindeer and moose CWD cases, their geographical distribution and epidemic curves based on raw cumulative observations (**Figure 10**) supports the notion that CWD in Norwegian moose is distinctly different from that in reindeer (Pirisinu et al. 2018). Interestingly, the Finnish and Swedish authorities have reported the same age-segment and tissue-distribution of PrP^{Sc} in their moose CWD cases as that of Nor-16CWD (Finnish Food Authority 2020, National Veterinary Institute 2020), suggesting that this is a similar if not identical type of CWD. Three of the CWD cases in Swedish moose displayed WB glycoprofile and PrP^{Sc} deposition patterns similar to Nor-16CWD (Ågren et al. 2021). The overall picture suggests that CWD in Europe could be categorized into two forms: CWD as in reindeer and atypical CWD as in moose (and red deer).

[16] Referred as cervid TSE

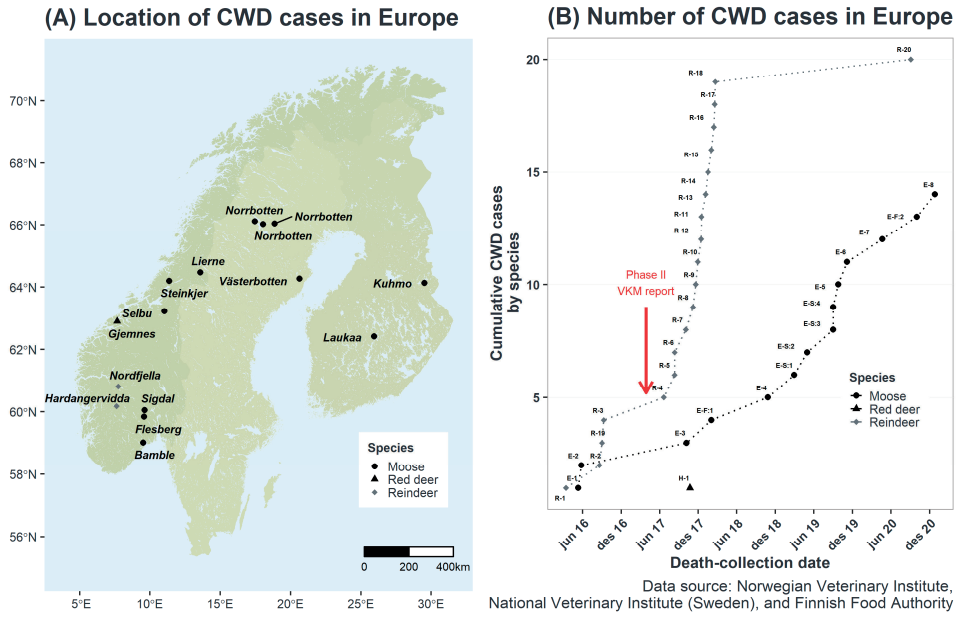

***Figure 10**. Data visualization of CWD in Europe (2016-2020). (A) Locations in Norway and Finland refer to the centroid of either a management area (reindeer) or a municipality (moose, red deer) from which one or more cases have been reported. Locations in Sweden refer to the coordinates of each case. (B) Cumulative number of CWD cases at each time a new case was registered as identified in **Table 3**. The legend color indicates either CWD form if described as contagious (gray) or atypical (black).*

Before CWD was discovered in Europe, the Republic of Korea reported the first cases outside North America, linked to the import of infected animals from Canada (Sohn et al. 2002, Kim et al. 2005). This outbreak and subsequent cases have been associated with the imported Canadian strain (Lee et al. 2013). The scenario differs in Europe as the origins of the circulating CWD strains are unknown (VKM 2016). CWD in Europe suggests a scenario of a host range distinct from that commonly found in North America being exposed to novel CWD strains (Nonno et al. 2020). These changes in the epidemiological triangle of CWD demand studies regarding the epidemiology of CWD in Europe including *PRNP* variability in the host. *PRNP* variation can change over geographical space and these spatial trends can be important to determine the innate susceptibility to CWD in recently exposed populations (Miller and Walter 2019). Studies of *PRNP* variability in Norwegian cervid populations are thus needed to assess the likelihood of CWD spillover over time (VKM 2017).

3 Aims

The principal aim of the investigations described in this PhD thesis was to get a better acquaintance of the genetic susceptibility to CWD by characterization of the prion protein gene (*PRNP*) variation in distinct cervid species potentially exposed to CWD agents following the outbreak of the disease in Norway.

Specifically, this thesis aims to study:

1. The non-genetic factors for susceptibility to CWD in Norwegian reindeer (article I).
2. *PRNP* variability in CWD cases from Norwegian reindeer and moose (articles II and IV)
3. The genetic association between CWD status and *PRNP* variability in Norwegian reindeer (article II).
4. The *PRNP* variability in an extensively distributed cervid species in Europe, the red deer (article III)
5. The *PRNP* variability in cervid populations exposed to novel CWD strains, Norwegian reindeer, red deer, moose, and roe deer (article IV)

4 Summary of articles

4.1 Host epidemiology of CWD in a novel geographical setting: from demography to *PRNP* genetics

4.1.1 **Article I:** The demographic pattern of infection with chronic wasting disease in reindeer at an early epidemic stage

Studies of CWD in North America have shown that age and sex are important for disease transmission. This could result from differences in pathogen exposure according to sex, social organization, age-group, and behavior. The CWD outbreak in Norwegian wild reindeer offered an opportunity to investigate whether similar effects were present in this highly social cervid. Article I describes and compares the demographic features of reindeer from Nordfjella zone 1 in Norway. This subpopulation was culled following diagnosis of CWD in March 2016. Complete records of sex and age were available for 2359 animals of which 19 tested positive for CWD PrP^{Sc}. Adult males were found to be 2.7 times more likely to test positive compared to adult females, and this hazard increased with age in males. DNA microsatellites were used to estimate the genetic relatedness among the 19 positive reindeer and compared with that of 41 control animals from the same population. Both genetic variation and relatedness estimators were similar between groups and no parent-offspring relationships were detected in case- or control- animals. It was concluded that sex and age categories are strong risk factors for CWD in reindeer. In the view of the low relatedness between case animals, and the higher hazard among males, the Nordfjella zone 1 outbreak is consistent with a horizontal mode of CWD transmission.

4.1.2 **Article II:** Chronic wasting disease associated with prion protein gene (*PRNP*) variation in Norwegian wild reindeer (*Rangifer tarandus*)

Article I revealed similarities between CWD in North America and that observed in Norwegian reindeer regarding some non-genetic risk factors in the host. It is well-established that variation in the *PRNP* gene can modulate CWD susceptibility and disease progression. Whereas a reduced susceptibility to CWD is associated with Ser138Asn, a marker for allele *138N_1226Q*, in orally challenged reindeer from North America, Norwegian reindeer lack this substitution, but harbors others not found in

North American reindeer. To investigate the genetic association between *PRNP* variability in Norwegian reindeer and CWD status, the *PRNP* profiles of 19 reindeer testing positive for CWD were compared with 101 age-matched controls that tested negative for CWD. All were derived from the reindeer population in Nordfjella zone 1. Specifically, three issues were addressed (1) *PRNP* variability was analyzed and reported as a whole sequence arrangement, (2) the study design was a matched case control-study on age category and sex to eliminate their effect as confounders (3) use of Firth logistic regression to account for the low CWD prevalence when investigating the association between disease status and *PRNP* genotype. Among the 19 cases and 101 controls, five *PRNP* alleles were identified, combining into 14 genotypes. A novel allele in reindeer encompassing an octapeptide deletion was identified (*deletion_226Q*). *PRNP* alleles *A* (*226Q* in this thesis) and *C* (*deletion_226Q* in this thesis) were more abundant in the case group than in the control group. The Firth regression model showed that animals carrying two copies of either alleles *A* (*226Q*) or *C* (*deletion_226Q*) had an increased chance of testing positive for CWD PrP^{Sc}. Therefore, genotypes *A*/*A* (*226Q*/*226Q* in this thesis) and *A*/*C* (*226Q*/*deletion_226Q* in this thesis) are considered to increase susceptibility to CWD in Norwegian reindeer.

4.2 The spatial trends of *PRNP* variability in a geographically novel CWD setting

4.2.1 **Article III:** Variation in the prion protein gene (*PRNP*) sequence of wild deer in Great Britain and mainland Europe

The CWD outbreak in Norway could potentially spill over into other cervid species in Europe over time. Experimental transmission of CWD PrP^{Sc} to red deer (Martin et al. 2009, Balachandran et al. 2010), Reeves' muntjac deer (Nalls et al. 2013), and fallow deer (Hamir et al. 2011) has been demonstrated. Before the naturally occurring cases in Norwegian reindeer, reindeer had been shown to be susceptible to CWD under experimental conditions (Mitchell et al. 2012, Moore et al. 2016). The experimental data described above suggests a potential susceptibility among various deer species in Europe. Nonetheless, *PRNP* variability has not been extensively characterized in European deer species. In article III, an international collaboration between Great Britain, Czech Republic, and Norway allowed for a multicentric survey on *PRNP* variability in wild red deer in Europe, incorporating previous reports from Spain (Pitarch et al. 2018), Scotland, and Italy (Peletto et al. 2009). Article III also described *PRNP* variation in five other deer species in Great Britain. By comparing the red deer

data from six European countries, five *PRNP* alleles of variable distribution were identified. Two of the alleles were unique to certain populations. The frequency of the major alleles $T_{98}P_{168}E_{226}$ (*226E* in this thesis) and $T_{98}P_{168}Q_{226}$ (*226Q* in this thesis) varied between countries. British red deer showed regional differences in *PRNP* variability. This could be attributed to the demographic history of the populations. *PRNP* was monomorphic in the remaining studied British species with the exception of Chinese water deer, which harbored three *PRNP* alleles, one of them $S_{100}\Delta_{71\text{-}78}$ comprised an octapeptide deletion encoding allele *C* (*deletion_226Q*) as in Norwegian reindeer. In the dataset from Great Britain, roe deer, Sika deer and Chinese water deer encode a common allele TPE (*226E*), whereas fallow deer and Muntjac deer distinctively harbored conspecific *PRNP* alleles. Considering the abundance of susceptible alleles TPE (*226E*) and TPQ (*226Q*), together with the lack of alleles associated with reduced CWD susceptibility, article III suggests that a large proportion of European deer species may be susceptible to CWD.

4.2.2 **Article IV:** Chronic wasting disease in Norway - a survey of prion protein gene variation among cervids

Following observation of CWD in reindeer, red deer, and moose in Norway, Norwegian cervids are therefore likely the most exposed European population to CWD PrP^{Sc}. Article IV investigates the *PRNP* variability of the four most prevalent Norwegian cervid species, sampled from different areas and subpopulations. For reindeer, the analysis involved 12 subpopulations of which seven were from wild reindeer management areas. Seven *PRNP* alleles were found in reindeer, two in red deer and moose, while roe deer were monomorphic. The four species encoded a common *PRNP* variant (*226Q*) associated with CWD susceptibility. Whereas *226Q* dominated in most species, allele *226E*, which is also associated with CWD susceptibility in North America, was the most frequent allele in red deer. No other species encoded *226E*. The distribution of *PRNP* variants was significantly different between wild and semi-domestic reindeer. Allele *A* (*226Q*) was present at high frequencies in wild reindeer, whereas allele *B* (*225Y_226Q* in this thesis) dominated in semi-domestic reindeer, suggesting that wild Norwegian reindeer might be more susceptible to CWD than semi-domestic. Spatial variation in *PRNP* genotype frequencies in red deer and moose was consistent with previous investigations of genetic structuring in these species in Norway. Article IV concludes that based on *PRNP* variability alone, Norwegian cervid species must be regarded as potentially susceptible to CWD.

5 Material and methodological considerations

5.1 Biological material

In this thesis, frozen brain from Norwegian reindeer, red deer, moose, and roe deer served as the biological material for genomic DNA isolation. The Norwegian Veterinary Institute (NVI) provided brain samples harvested between May 2016 and September 2018. Genomic DNA was isolated at high yield with the DNeasy Blood & Tissue kit, following the manufacturer's protocol. Sample collection in wild cervids involved both trained professional and recreational hunters, which resulted in samples of variable quality, in part due to autolysis (Viljugrein et al. 2019). After examination for CWD PrP^{Sc}, all samples were stored frozen (-20°C). The DNeasy Blood & Tissue kit is suitable for frozen animal tissues and yields optimal quality DNA even from decomposing corpses (Uerlings et al. 2021), thus bypassing previously reported limitations in the genetic analysis of a CWD case caused by tissue degradation (Kreeger et al. 2006).

Additional genomic DNA from reindeer was provided by the Medical Genetics Unit, Department of Preclinical Sciences and Pathology (PREPAT) at NMBU and by the NVI. The DNA material from NMBU corresponded to wild reindeer hunted between 2005 and 2013, while that from the NVI originated from cervids tested positive for CWD PrP^{Sc}. All genomic DNA from Norwegian cervids was subjected to the same downstream analysis using the same standardized workflow as described in articles I-IV.

In article III, small sections of ear-tips from cervids from Great Britain and Czech Republic were available for DNA extraction, which complemented a large collection of archived genomic DNA from British species. The preparation of ear-tip DNA is discussed in article III.

5.2 Study populations

Norwegian cervids represent the source population for articles I-IV. Article III included populations from England, Scotland, and the Czech Republic (n=1069) in addition to red deer from Norway (n=50). In articles III and IV, the study population from each country aimed to include samples from different geographical regions. When possible, the selection criteria also considered the species genetic structure as

determined by neutral markers. The Czech red deer in article III consisted of the Western and Eastern lineages described in Central Europe (Krojerová-Prokešová et al. 2015), and Norwegian red deer and moose in article IV comprised animals from genetically differentiated subpopulations (Haanes et al. 2010, Haanes et al. 2011). The small number of studied animals compared to the overall population was a limiting factor, yet randomization in the sample selection addressed potential bias associated with small sample size. The similarities between population structure and spatial patterns of *PRNP* variation supports the representativeness of the genetic variation in the studied populations.

Articles I and II specifically focused on the northern territory of Nordfjella (**Figure 10**), referred to as zone 1, where all CWD positive reindeer (*n*=19) had been identified at the time of publication. It should be emphasized that reindeer in Nordfjella zone 1 were culled between March 2016 and May 2018. In article I, for all animals used to test for age- and sex- associations with CWD infection, complete records of CWD status, age class and sex (*n*=2365) were available. Differences in genetic relatedness (article I, compared to *n*=41) and *PRNP* variability (article II, compared to *n*=101) between the 19 CWD cases identified were compared with PrP^{Sc} negative reindeer from the same population. This allowed testing for explanatory variables acting on the likelihood of CWD infection in a reindeer subpopulation definitely exposed to CWD PrP^{Sc} with animals of different disease status.

5.3 DNA analysis

All DNA analyses of Norwegian species were based on PCR amplification and Sanger sequencing technology. The target sequences included 18 microsatellite loci (article I) and the coding sequence of the *PRNP* gene (articles II-IV). This thesis used an automated version of Sanger sequencing known as cycle sequencing. In short, these technologies rely on DNA extension and termination. In cycle sequencing, the process takes place during thermal cycling. The extension products incorporate fluorescent dye labels either as labeled primers (microsatellite analysis) or as labeled dideoxynucleotides (gene variant calling as for *PRNP*). By capillary electrophoresis, the extension products of various length are sorted by size and a laser beam causes their dye label to fluoresce. The extension products were scored by length size on Applied Biosystems 3500xL Genetic Analyzer (RRID:SCR_019574). The analyzer converts the fluorescent signal into digital data which was analyzed by specialized software: GeneMapper (RRID:SCR_014290) version 5.0 for microsatellites analyses in article I and SeqScape (RRID:SCR_001604) version 3.0 for *PRNP* variant calling in articles II-IV.

5.3.1 DNA fragment analysis: microsatellites

DNA microsatellites are appropriate genetic markers for studies of genetic structure and kinship. Microsatellites, also referred to as short tandem repeats (STR), are sequence repeats of one to six nucleotides scattered through the genome. The genetic variation in microsatellites derives from changes in the number of repeats, which can vary significantly, and most microsatellites are thus, highly polymorphic. Microsatellites are commonly localized in non-coding DNA and are thus used as neutral genetic markers (Ellegren 2004).

The 18 microsatellite loci used in article I had previously been found to be highly polymorphic, codominant inherited, reliably scored in reindeer and suitable for multiplexing (Swarbrick et al. 1992, Buchanan and Crawford 1993, Bishop et al. 1994, Wilson et al. 1997, Røed 1998, Røed and Midthjell 1998, Jones et al. 2000, Yannic et al. 2014). When analyzing several loci in the same animal, multiplex PCR is advantageous as it is cost effective, time saving and provides increased data accuracy (Guichoux et al. 2011). Therefore, the multiplexing of the 18 microsatellite loci as described and used in article I provided resolution satisfactory for distinguishing individuals in a closely related subpopulation of reindeer in Nordfjella zone 1. In addition, this resolution was sufficient to discriminate parent-offspring relationships as tested for in article I.

5.3.2 DNA sequencing: gene variant calling

In the search for gene variants of medical importance, two approaches are commonly used: (a) the hypothesis-driven and, (b) hypothesis-free approaches (Kitsios and Zintzaras 2009, Licinio 2010). The hypothesis-driven approach is particularly useful in diseases with a strong genetic determinant, as is *PRNP* for CWD. In this approach, genetic variation can be investigated by Sanger sequencing. On the other hand, the hypothesis-free approach employs high throughput sequencing to explore the whole genome, allowing for the objective identification of putative risk loci for the condition of interest. Seabury et al. (2020) used this approach in a genome-wide association analysis for a large cohort of CWD-affected and non-affected white-tailed deer. This study found evidence that loci other than *PRNP* may affect CWD susceptibility, although *PRNP* variability was the locus with the largest effect. In the study of CWD in Europe, characterization of *PRNP* heterogeneity is the first step for understanding CWD susceptibility in the region. This thesis uses the Sanger sequencing principle as it is still the most accurate, gold standard technology. While it is restricted to a maximum length of 1000bp, this fitted perfectly analysis of the *PRNP* coding sequence in cervids (771bp) (Petersen et al. 2017). The coding sequence of *PRNP* was PCR

amplified using primer sets targeting upstream (intron 2) and downstream (exon 3) flanking regions (**Figure 3**) as described in articles II-IV. The expected PCR products ranged between 818-1082 bp. Sequencing of the amplicons was performed with either the same PCR primers (articles II and IV, Norway in article III) or a specific sequencing primer (article III). Sequence alignment and manual inspection was conducted in MEGA Software (RRID:SCR_000667) version 7.0.26. All *PRNP* sequencing analyses in this thesis targeted the coding sequence.

5.3.2.1 Variant calling: SNV vs SNV complex: the *PRNP* allele

As discussed in the introduction, most *PRNP* variants identified in cervids vary at a particular codon featuring a distinctive amino acid (**Figure 6**). Therefore, traditional genotyping strategies based on a single variable codon may fail to identify other *PRNP* variants present in the population and consequently their contribution to the outcome of interest. Haley et al. (2019) addressed this issue in white-tailed deer by including all variable codons in their study population. Their findings strengthened that allele *96S_226Q* significantly reduces CWD risk and that less frequent alleles (i.e. *95H_226Q* and *116G_226Q*) had a similar effect, which could have gone unnoticed using a single codon (codon 96) strategy, since all encode Gly96. Statistical comparisons can be easier when considering a single variable codon. Nonetheless, when considering multiple codons in close linkage, the DNA arrangement in a chromosome can be predicted and this type of analysis provides more power to assess genetic associations (Schaid 2004). Therefore, *PRNP* alleles in this thesis were analyzed as a linear amino acid arrangement, with two *PRNP* alleles per individual expressed as *PRNP* genotypes.

The *PRNP* alleles were inferred using a three-step approach for each species (Spooner et al. 2018): (1) samples were aligned to the reference sequence for each species i.e. the most frequent *PRNP* variant across multiple studies; (2) variants were called by identifying variable codons compared to the reference sequence; (3) amino acids at variable codons were assigned into separate *PRNP* alleles by using linkage disequilibrium analysis, with two *PRNP* alleles generated for each individual. The linkage disequilibrium analyses as described in articles II and IV were done using the *genetics* package in R (RRID:SCR_001905).

In research data management, interoperability is an important principle to integrate data. The HGVS nomenclature are recommended for uniform and unequivocal description of sequence variants in humans, but can be applied to all species (den Dunnen et al. 2016). The basic principle relies on explicit definition of the reference sequence for residue numbering, reporting of changes at the nucleotide and protein

levels and use of an unequivocal description. The importance of this initiative is illustrated in how differently *PRNP* variation is reported among cervids. A simple example is allele *226K* in white-tailed deer, which has been termed as either haplotype K, allele QGAK or Q226K. Many other examples become evident in section 2.3.1.2. In the absence of a proper reference sequence, an accurate data interpretation is limited to the variable codons the author decides to report (Mitchell et al. 2012, Cheng et al. 2017); any other assumptions would be 'best guess'. The main challenge would arise for comparisons between populations harboring distinct variable codons. Moum et al. (2005) found a strong association between Nor98/atypical scrapie in Norway and an allele with a substitution at codon 141 i.e. allele $AF_{141}RQ$, a variant position commonly not reported in sheep (**Figure 4B**). Comparability with similar association-studies in Germany and France was limited by the lack of information on codon 141 in the latter studies. The same challenge occurs in cervid populations e.g., Norwegian reindeer were shown to be variable at codons 176 and 225 (Wik et al. 2012), yet data on these codons could only be accurately compared with some studies from North America (Happ et al. 2007, Moore et al. 2016). The occurrence of a 24 bp deletion in *PRNP* among Norwegian reindeer (articles II and IV) reinforced the need to follow a standardized nomenclature[17] to avoid confusion when reporting sequence variants.

The HGVS nomenclature appears infrequently used in the literature for reporting *PRNP* variation. However, there are many benefits arising from using a standardized system, especially when different species are compared. To find a balance between the current trend and a more standardized approach, articles II and IV use a simple nomenclature based on the variable codon e.g. *B* (Tyr225) connected to that recommended by the HGVS nomenclature guidelines e.g. DQ154293.1:c.[674C>A] for nucleotide and AAZ81474.1:p.[(Ser225Tyr)] for protein description.

5.4 Statistical analyses

5.4.1 Hardy-Weinberg equilibrium

A rule of thumb in molecular genetic studies is to test for Hardy-Weinberg equilibrium (HWE). HWE is a theoretical law based on an expected relationship between allele and genotype frequencies (Guo and Thompson 1992). These

[17] e.g. 3' rule is a standard to arbitrary localize variants in stretches of repeated DNA to the most 3' position possible

expectations should be fulfilled if specific assumptions are met. Deviations from HWE may indicate failure in one or more assumptions and possible scenarios include non-random mating, population stratification (Balding 2006), but can also suggest genotyping errors (Salanti et al. 2005). For instance, the pooled Norwegian reindeer populations were not in HWE at *PRNP* (article IV) which agrees with the main genetic structuring of this population into wild and semi-domestic.

In case-control genetic association studies as for article II, inferences may be compromised if the genetic data is not in HWE. Therefore, a routine test of HWE must be performed in appropriate controls. The genotype frequencies in disease-free controls in article II were in HWE, suggesting there was little alleles bias in the population that may have affected the validity of the study (Salanti et al. 2005).

5.4.2 Genetic relatedness

Pairwise relationships between individuals can be estimated using molecular markers such as microsatellites. This is particularly useful in populations lacking a pedigree record as is the case for wild populations Different methods for the estimation of pairwise relatedness are available. Here, Lynch and Ritland's method was chosen (article I) for its computational simplicity and robustness, particularly when many loci are used (Lynch and Ritland 1999). The genetic relatedness analysis as described in article I was performed using the *related* package in R (RRID:SCR_001905).

5.4.3 Case-control genetic association

Case-control association studies assume the differences in the genetic variation between cases and controls are due only to disease status, excluding any differences in genetic background (Lewis 2002). Therefore, the importance of HWE testing.

The study design for article I was of a matched case-control because age category and sex are variables with a known effect on CWD outcome (article I) and may act as confounders. The genetic differences between cases and controls can be tested by different methods including tests of independence and logistic regression. In article I, two categorical variables: disease status and *PRNP* variability (allele, genotype) were tested for independence using Fisher's exact test. Odds ratios of testing CWD positive based on the *PRNP* genotype were estimated by Firth logistic regression. Article II followed the STrengthening the REporting of Genetic Association studies (STREGA) recommendations to provide a more comprehensive and transparent report (Little et al. 2009).

5.4.4 Other associations

In this thesis, most comparisons involved two categorical variables for which tests of independence and regression models were a constant analysis methodology. Features of the datasets dictate which methods should be considered. The association between CWD outcome (dependent variable) and non-genetic factors such as age category and sex were analyzed by logistic regression, whereas the hazard of being CWD positive (dependent variable) as an effect of age category and sex was analyzed by Cox regression (article I). Throughout this thesis, associations between *PRNP* variation and spatial distribution were also explored. The frequency of *PRNP* variants between geographical locations, regions and countries were compared either by Chi square test (article III) or Fisher's Exact test (article IV). For article IV, the Bonferroni correction method was employed to adjust the *P*-value for multiple comparisons. In addition, article IV evaluated the association between the occurrence of *PRNP* genotypes in red deer and moose and geographic coordinates, which was modelled using a multinomial logistic regression.

6 General discussion

6.1 Non-genetic and genetic factors associated with CWD in Norwegian cervids

The CWD scenario in Europe differs from that in North America in various aspects. CWD in Europe manifests not only in its contagious form as described in North America, but also in unusual, potentially sporadic forms, generically referred to as "atypical". Studies in bank voles have evidenced that the European CWD forms are caused by different strains, distinguishable from those causing CWD in North America (Nonno et al. 2020). Therefore, control strategies in Europe should be based on the epidemiology of European CWD strains (Mysterud et al. 2021).

When aiming to identify risk factors for CWD, many researchers have found disease propagation to largely depend on host contact with the disease source (Rees et al. 2012) and on host *PRNP* genetics (Johnson et al. 2006, Kelly et al. 2008, Brandt et al. 2015). Wild Norwegian reindeer, the species affected by contagious CWD in Europe, have a highly social and nomadic behavior in addition to *PRNP* alleles not found in other species with a longer record of CWD. Therefore, Norwegian reindeer have host factors distinguishable from those commonly described for CWD in North America and therefore need to be studied in the context of emergent CWD in Europe. The demographic pattern of CWD infection in reindeer shows that the disease is sex-biased, is more likely in adult males and that infection probability increases with age in adult males (article I). This disease pattern is similar to that described in mule deer and white-tailed deer (Rees et al. 2012). Whereas mule and white-tailed deer show matrilineal grouping, which allows effective contact for CWD transmission among kin females, reindeer groups comprise both related and non-related individuals. CWD cases in Norwegian reindeer manifest in relatively unrelated animals, as affected females are too young to parent most cases and that a lack of parent-offspring relationships was identified amongst cases (article I). Together with the low prevalence, these data are consistent with CWD in Norwegian reindeer representing an early stage epidemic based on horizontal transmission via direct contact between non-related individuals.

Among the CWD cases reported in Norwegian moose and red deer, no epidemiological link has been established and most were geographically separated (**Figure 10**), making horizontal animal to animal spread from a single outbreak unlikely. Nor-16CWD, as CWD in moose was denominated (Pirisinu et al. 2018),

shares important features with sporadically occurring atypical forms of prion disease, such as Nor98/atypical scrapie in sheep and atypical H and L forms of BSE in cattle. Genetic data on atypical prion diseases in other species including humans and other ruminants indicates that homozygosity of certain alleles may increase the CWD risk (Palmer et al. 1991, Benestad et al. 2003, Casalone et al. 2004, Moum et al. 2005, Mead et al. 2011). All atypical CWD cases in Norwegian moose and red deer reported by Vikøren et al. (2019), Pirisinu et al. (2018) and in article IV are homozygous (**Table 4**). Cumulative data suggest that CWD animals homozygous for susceptible alleles (*226Q* or *226E*) have a faster disease progression than heterozygous animals (Haley et al. 2019, Haley et al. 2020). Despite dissimilarities in the epidemiology of CWD in its different forms, *PRNP* heterozygosity may be an independent susceptibility-reducing element.

***Table 4**. PRNP genotype (protein level) in Norwegian cervids.*

Species	*n*	*PRNP* genotype	In article	Location in Norway
CWD				
Reindeer	10	*226Q/226Q*	*A/A*	Nordfjella 1
	4	*226Q/225Y_226Q*	*A/B*	Nordfjella 1
	4	*226Q/deletion_226Q*	*A/C*	Nordfjella 1
	1	*225Y_226Q/deletion_226Q*	*B/C*	Nordfjella 1
	1	*226Q/176D_226Q*	*A/D*	Hardangervidda
Atypical CWD				
Moose	6	*226Q/226Q*	*109K/109K*	Selbu, Lierne, Steinkjer and Bamble
	2	*109Q_226Q/109Q_226Q*	*109Q/109Q*	Flesberg and Sigdal
Red deer	1	*226E/226E*	*226E/226E*	Gjemnes

6.2 *PRNP* variation in cervids from Norway

PRNP amino acid sequences across Norwegian reindeer, red deer, and moose differed at ten variant positions, all of which were conspecific (**Figure 11**). Nonetheless, the four species shared allele *226Q* which was differently distributed in these species (article IV).

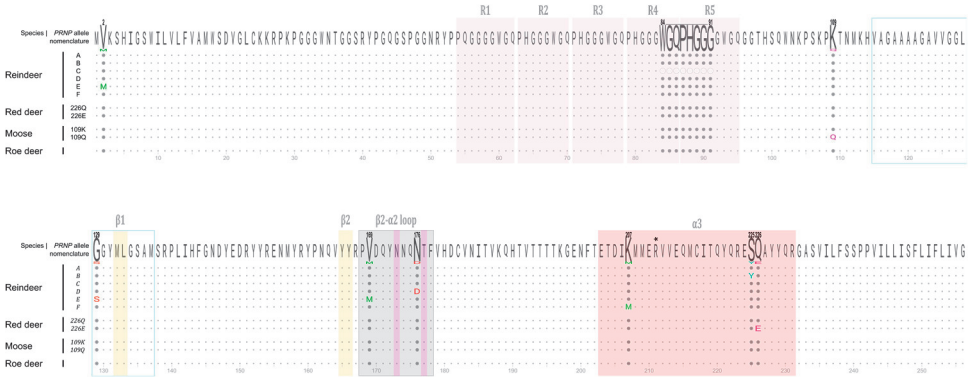

Figure 11. *PRNP alleles (protein level) identified in Norwegian cervids. Variant codons are highlighted. Peptide repeat region (shaded pink) and hydrophobic region (framed cyan) are highlighted in the non-globular domain. β strands (shaded yellow), β2-α2 loop (shaded grey) and α3 helix (shaded red) are based on the 226Q structural model of mule deer (6FNV). Characteristic amino acids in cervid PrP^{C} associated with the β2-α2 loop (shaded gray based on 226Q) are shaded fuchsia.*

Reindeer with two copies of allele *226Q* (i.e. allele *A* in article II) appeared to have increased CWD risk. Similar *PRNP* genotypes were observed in red deer (i.e. *226Q/226Q* in article IV), moose (i.e. *109K/109K* in article IV) and roe deer (i.e. monomorphic in article IV), implying potential susceptibility to the contagious CWD described in Norwegian reindeer.

The co-existence of *PRNP* variants different than *226Q* in the various species might also impact CWD dynamics. Challenge studies testing the effect of these *PRNP* genotypes on CWD susceptibility (disease status, stage) could shed light on the role less common genotypes might play in managing CWD (Haley et al. 2019). In the meantime, *in vitro* assays provide some clues on some of the alleles observed in Norwegian reindeer.

By using RT-QuIC, Haley et al. (2017) have shown that recombinant PrP encoded by either *225Y_226Q* (named 225Y) or *2M.129S.169M_226Q* (named SSM) was less efficient than *226Q* (named QGAQ) for initial PrP^{C} to PrP^{Sc} conversion. These findings parallel data on orally challenged reindeer that showed that *226Q/226Q* animals (identical to mule deer) succumbed to CWD, whereas animals carrying either alleles *138N_226Q* or *2M.129S.169M_226Q* remained disease free (Mitchell et al. 2012). To what extent these reduced susceptibility data apply to Norwegian cervids is uncertain considering the strain differences between those in Norway and North America (Nonno et al. 2020). The observations of increased CWD risk in Norwegian reindeer

carrying allele *226Q* (article II) complies well with these data and from mule deer (Brayton et al. 2004, Wilson et al. 2009) and white-tailed deer (Johnson et al. 2006), suggesting that the allele *226Q* confers high susceptibility towards different CWD strains.

6.2.1 Molecular impact

The localization of substitutions in either the structured or unstructured domains of PrP^{C} might dictate different mechanisms by which these affect PrP^{C} to PrP^{Sc} conversion (Angers et al. 2014). Additionally, we should consider that proteolytic processing in PrP^{C} may occur under physiological and pathological conditions at conserved cleavage sites, releasing well-defined fragments (Linsenmeier et al. 2017). *PRNP* substitutions might disrupt recognition sites for the proteases involved in PrP^{C} cleavage. Additional sites have been proposed for some of these proteases, suggesting a process far more complex and dynamic than previously thought (McDonald et al. 2014) and that the possibility of species differences in the PrP^{C} – protease interaction should also be considered. Among Norwegian cervids, substitutions were found in codons localized in both domains of PrP^{C} (**Figure 11**).

Substitutions in the flexible tail of PrP^{C} were found in reindeer (allele *C* i.e. *deletion_226Q*) and moose (allele *109Q* i.e. *109Q_226Q*), in proximity of the β- and α-cleavage sites respectively. Trp84_Gly91del as observed in allele *deletion_226Q* signifies loss of an octapeptide in the repeat region, apparently without affecting PrP^{C} processing, with the exception of the expected length difference in PrP^{C} (article II). On the other hand, Lys109Gln as observed in allele *109Q_226Q* seems to confer a different PrP^{C} processing pattern, probably due to its proximity to the α-cleavage site (upstream the hydrophobic region) (Kallunki Nyström 2019). If the fragments resulting from these substitutions have distinguishable physiological or pathological effects is about to be determined.

Substitutions in the structured globular domain of PrP^{C} were predominantly observed in reindeer. Most of these substitutions occur either at the beginning of the globular domain, at the β2-α2 loop or the C-terminal end of α3 helix (compare **Figure 11** with **Figure 4A**). Substitutions at these spots might impact the long-range interactions mediating the plasticity of the so-called PrP^{Sc} conversion epitope. For instance, Angers et al. (2014) modelled the structural effect of Ser225Phe on *226Q* (*225F_226Q*) compared to *226Q* and *226E*. The substitution resulted in conformational changes that allowed for hydrogen bonding between Asp170 and Tyr228 (**Figure 11**), which stabilized the interactions between β2-α2 loop and the C-terminal end of α3 helix (**Figure 5**). Ser225Tyr as in *225Y_226Q* might have a similar

effect considering the physicochemical similarities between phenylalanine and tyrosine. Norwegian reindeer harboring the genotype *225Y_226Q/225Y_226Q* (i.e. genotype *B/B* in article II) being at a significant lower CWD risk than those with *226Q/226Q* (i.e. genotype *A/A* in article II), fits well with structural modelling (Angers et al. 2014) and *in vitro* features of *225Y_226Q* (Haley et al. 2017) that suggest *225Y_226Q* is an allele awarding reduced susceptibility to CWD. Substitutions at the beginning of the globular domain in human PrP^C such as Gly127Val and Met129Val (residues 130 and 132 in cervids, **Figure 4B**) appear to account for conformational changes explaining their reduced susceptibility in human prion diseases (Zheng et al. 2018, Hosszu et al. 2020). In humans, Gly127Val (residue 130 in cervids) allows for interaction between Met166 and Tyr218 (codons 169 and 221 in cervids) in the human PrP^C harboring this substitution (compare in **Figure 4B**). In the cervid PrP^C encoding Gly129Ser and Val169Met (allele *2M.129S.169M_226Q*, i.e. allele E in article II, IV), a similar interaction between the emergent Met169 and the constitutive Tyr221 (codons 166 and 218 in humans) might occur, allowing interaction of the β2-α2 loop region with the α3 helix (see allele E in **Figure 11**). Yet, other innate structural differences between human and cervid PrP^C might also alter intramolecular interactions (**Figure 4B**).

Norwegian red deer harbored a Gln226Glu substitution resulting in *226E*. The differences between the Norwegian and North American strains may challenge conventional interpretation of susceptibility associated with allele *226E*. *In vitro* assays have shown that "deer" PrP (*226Q*) compared to elk PrP (*226E*) is a more efficient substrate for detection of PrP^{Sc} in infected Norwegian reindeer brains (Bistaffa et al. 2019). If allele *226Q* compared to *226E* is more susceptible to misfolding induced by the CWD strain observed in Norwegian reindeer, it can be hypothesized that the dominance of *226E* among sympatric red deer might affect the spread dynamics in this species.

In summary, the substitutions characterizing the *PRNP* alleles in the different Norwegian species may arise from changes in PrP^C processing and intramolecular (possibly also intermolecular) interactions that could also influence CWD dynamics in Norway.

6.3 Potential factors affecting *PRNP* variation

As previously indicated, changes in *PRNP* sequences follow species phylogeny. *PRNP* variability within the *Cervidae* parallels evolutionary relationships (**Figure 7**). Within-species evolution also appears to be important as illustrated by the amount of *PRNP* variation between Norwegian cervids (article IV), corresponding well with

diversity patterns obtained with neutral genetic markers (Røed 1998). The differences in the distribution of *PRNP* alleles among these populations in Norway, particularly the marked contrast between wild and semi-domestic reindeer, raise questions about the factors affecting variability within *PRNP* coding sequences. Miller and Walter (2019) who studied the spatial heterogeneity of this gene among white-tailed deer in a region with emerging CWD in the USA, underlined the importance of understanding the driving forces behind *PRNP* variation for disease management.

Adaptive processes have been suggested as possible causal factors for the differences in *PRNP* variability. Nevertheless, there is no consensus on the acting form of selection. For instance, it is considered that the function of most protein-coding genes is maintained by preserving their gene structure, by means of purifying selection (Nei et al. 2010). This principle would apply to *PRNP* considering its conserved structure (Robinson et al. 2012). However, this implies that most non-synonymous substitutions would be eliminated by purifying selection, which can be argued may be rather common. Met129Val in human *PRNP*, for instance is found in a range of populations worldwide and this could be explained by means of balancing or positive selection (Soldevila et al. 2006). Similarly, selection favoring less susceptible *PRNP* genotypes i.e. heterozygous carriers of *96S_226Q* or *132L_226E* is recorded in CWD exposed populations of free-ranging white-tailed deer and elk (Robinson et al. 2012, Monello et al. 2017). By epidemiological modelling, the increment of such genotypes was consistent with an adaptive response of *PRNP* to CWD PrP^{Sc} and was referred to as disease/pathogen-driven selection (Robinson et al. 2012, Monello et al. 2017). A similar effect might apply to the "scrapie signature" in sheep from scrapie-affected flocks, where susceptible genotypes in aged animals become very scarce if not absent (Baylis et al. 2000). However, these patterns may vary according to the strain or *PRNP* genetic composition of the host population.

For instance, Miller and Walter (2019) found some white-tailed deer subpopulations where the most susceptible genotypes in codons 95 and 96 were directly proportional to infection rates, which is not consistent with a pathogen-driven selection model. The spatial fluctuation in *PRNP* variability registered by Miller and Walter (2019) was independent of infection rate and may partly be explained by demographic factors such as dispersal and population structure. This thinking may be reasonable for understanding *PRNP* heterogeneity in populations without a known CWD record. Certainly, distinctive frequencies of *138N_226Q* have been found in North American caribou (Cheng et al. 2017, Arifin et al. 2020), whereas this allele is relatively scarce in European Russia (Kholodova et al. 2019) and absent in Scandinavia (Wik et al. (2012), article IV). Similarly, around half Alaskan moose

harbored *209I_226Q* (Huson and Happ 2006), an allele absent in Scandinavian moose which has *109Q_226Q* as the most common allele (Wik et al. (2012), article IV). In both cases, these dissimilarities could somehow parallel the different origins of American and European populations of *Rangifer* and moose (Hundertmark et al. 2002, Yannic et al. 2014). Regional differences in *PRNP* variants appear at even finer scales in both affected (Jewell et al. 2005, Brandt et al. 2018, Miller and Walter 2019) and non-affected cervid populations (Peletto et al. 2009, Pitarch et al. 2018). Altogether, distribution of *PRNP* variants based on their geographical location likely follows the demographic history of the populations.

In this sense, comparisons between *PRNP* variability and the genetic structure as determined by neutral genetic markers may enlighten a plausible demographic effect. Red deer stand out as a widely distributed species inhabiting many countries in Europe (Linnell et al. 2020). Available data on their genetic structure (Zachos et al. 2016), gives evidence of a *PRNP* distribution roughly corresponding to their population structure (article III). This strengthens the impact demographic factors may have in *PRNP* heterogeneity. An example is the novel substitution Ile247Leu that is restricted to Czech Republic, more specifically to red deer belonging to the Western lineage but not the Eastern lineage also present in Central Europe (Skog et al. 2009). Another example includes the few *PRNP* variants in Norwegian red deer (article III), which is a population distinguished by its lower diversity compared to other populations in Europe (Zachos et al. 2016). The observations of *PRNP* variants in Norwegian reindeer, red deer and moose having a spatial distribution (article IV) concordant with their genetic structure (Haanes et al. 2010, Haanes et al. 2011, Kvie et al. 2019) also supports the demography hypothesis.

Norwegian red deer and moose are continuously distributed species with genetically distinguishable subpopulations separated by long and wide fjords (**Figure 9**). This characteristic spatial distribution is also reflected in *PRNP* frequencies in these subpopulations compared to the main population (article IV). For Norwegian reindeer, the division into wild and semi-domestic populations highly influences *PRNP* variability, probably related to the different origins suggested for these populations (Røed et al. 2008, Røed et al. 2018).

On the other hand, the behavioral dissimilarities between wild and semi-domestic reindeer populations might explain the trade-off between *PRNP* alleles. Wild and semi-domestic reindeer have discrepant vigilant and fright responses (Reimers et al. 2012) and PrP^{C} has been ascribed a plausible role in stress adaptation (Nico et al. 2005). The *PRNP* differences between these populations may thus act as a reasonable explanation. However, most bioassays addressing the effect of *PRNP* in animal

behavior have compared the processes associated with expressing or not expressing PrP^C (Nico et al. 2005, Le Pichon et al. 2009, Schmitz et al. 2014). Interpretation of the effect of diverse PrP^C conformers is therefore limited. Yet some studies have shown Met129Val in humans to apparently influence some learning abilities (Rujescu et al. 2003, Papassotiropoulos et al. 2005), including visuospatial memory (Houlihan et al. 2009). Hypothetically, the *PRNP* variants predominant among semi-domestic reindeer could be related to their reduced vigilance, alert, and fright responses, and thus to a selection process related to reindeer husbandry.

Earlier, it was discussed whether changes in the apportion of *PRNP* variants may result from an adaptive response to CWD (Robinson et al. 2012, Monello et al. 2017). Meaning the proportion of "resistant" *PRNP* genotypes increases in CWD exposed populations, dependent mainly on the intensity of PrP^{Sc} exposure. In the case of Norwegian reindeer, the major exchange between wild and semi-domestic reindeer involves alleles *226Q*, *225Y_226Q* and *2M.129S.169M_226Q* (A, B and E in articles II and IV) among which allele *226Q* associates with an increased CWD risk. Observations on *225Y_226Q* and *2M.129S.169M_226Q* (B and E in articles II, IV) point towards a reduced susceptibility to PrP^{Sc} conversion, yet the current data is still scarce. Also importantly, prion strains may impose distinguishable selective regimes on *PRNP* substitutions (Moum et al. 2005) that may modulate pathogen-driven selection. Thus, what are regarded as susceptible genotypes for a particular strain may not apply for a different strain and alter the selective advantages of certain genotypes.

Nonetheless, if alleles *225Y_226Q* and *2M.129S.169M_226Q* (B and E in articles II, IV) show sustainable evidence to be less susceptible to the CWD strain in Norwegian reindeer, the semi-domestic population could be regarded as harboring a high proportion of less susceptible genotypes. Could this be a consequence of a historical unrecorded CWD outbreak in the semi-domestic reindeer ancestors? In this hypothetical scenario, if the wild reindeer population is regarded as an originally uninfected population, the comparative doubling of allele *225Y_226Q* (allele B in articles II, IV) frequency in the semi-domestic population should imply a very long history of CWD exposure (Robinson et al. 2012, Monello et al. 2017). Even with a potential "low susceptibility" conferred by the *PRNP* genotypes, CWD would likely be maintained at endemic levels (Robinson et al. 2012) implying without disease management, that prevalence should have increased over this considerable time. Therefore, animals at an advanced stage of disease either with clinical signs or with detectable PrP^{Sc} in the *medulla oblongata,* should have been found. The variability in disease progression between individuals challenges the certainty of CWD absence in

populations, particularly during epidemic growth (Viljugrein et al. 2019), which would not fit the hypothetical exposure in semi-domestic reindeer.

Testing for CWD PrP^{Sc} in semi-domestic reindeer started in 2002, with ~1000 animals analyzed and tested negative between 2002-2015 (Våge et al. 2020). It is true that this sampling regime allows for the disease to remain undetected, considering the very small sample size (compared to the real population and surveillance period) and use of a biological sample not suitable for early detection (Viljugrein et al. 2019) during this period (Våge et al. 2020). Nevertheless, between 2016-2019, the number of semi-domestic reindeer tested rose to 38 000, with all testing negative. Even though it could be hypothesized that CWD could have been present among semi-domestic reindeer at undetectable levels, this infection rate would have been too low to exert substantial changes in *PRNP* genetic makeup as observed in semi-domestic reindeer compared to wild reindeer. On the other hand, although at these levels CWD may exert appreciable changes in *PRNP* allele frequencies, it seems unlikely that infected animals would have remained unnoticed either by reindeer herders or CWD surveillance.

It is uncertain what the exact driving forces shaping *PRNP* variability among Norwegian cervids are. However, the number of *PRNP* variants argues against purifying selection. In a similar way, *PRNP* variation in humans is inconsistent with neutral expectations, showing geographic stratification; yet there is uncertainty on plausible causes, ranging from various types of selection to genetic drift (Soldevila et al. 2006). Understanding the factors affecting *PRNP* variation might be useful to develop long-term and feasible policies to control CWD spread.

6.4 Are less susceptible *PRNP* alleles the best choice?

Scrapie eradication programs have exploited the known genetic associations between *PRNP* genotype and disease susceptibility. Such experience can be useful in understanding some of the pros and cons of selective breeding based on *PRNP*. In sheep, susceptibility to classical scrapie is ascribed to the variability at codons 136, 154 and 171 (**Figure 4**). The amino acids at each of these codons are listed in numerical order to describe the *PRNP* alleles in sheep (e.g. allele VRQ). Sheep harboring VRQ/VRQ are the most susceptible, whereas those that harbor ARR/ARR show the greatest resistance to classical scrapie, setting the targets for the selective breeding: increase allele ARR and eliminate VRQ (Baylis and McIntyre 2004). Certainly, identification of Nor98/atypical scrapie challenged this knowledge when Moum et al. (2005) observed other alleles (AHQ, $AF_{141}RQ$) strongly associated with the atypical strain of scrapie. As Baylis and McIntyre (2004) clearly stated, breeding

programs are built on the assumption that "resistant" animals really are and will remain resistant. Could this apply to CWD susceptibility and *PRNP* variability in cervids?

So far, the cumulative data on CWD establish *226Q* and *226E* as the most susceptible *PRNP* alleles in cervids. However, other variants are also reported which indicates that they may be less susceptible but not resistant to CWD. It may be that the most susceptible *PRNP* alleles are disadvantageous for species survival but their persistence could be advantageous in perpetuation of heterozygous individuals (Martin et al. 2009), known to have a lower risk of CWD (Haley et al. 2019, Haley et al. 2020). Despite that CWD strains in Norway have been shown to be different from those in North America (Nonno et al. 2020), the disease phenotype in Norwegian reindeer largely resembles that traditionally observed in CWD in regard to clinical signs, pathological distribution and genetic susceptibility in the host (article II). In contrast, Norwegian moose manifest the disease in an atypical form (Pirisinu et al. 2018) that resembles many features of Nor98/atypical scrapie and due to its few cases, no genetic-associations can be drawn.

PRNP alleles associated with a known reduced susceptibility to CWD may have been identified because they reduce the risk of being CWD positive, but in disease management other factors should be considered. These include how these alleles may impact animal fitness, prion shedding, incubation time (Haley et al. 2019) and adaptability to novel strains (Duque Velásquez et al. 2020). Data on the effect of *PRNP* variability on cervid fitness is still limited and controversial (Wolfe et al. 2014); but it appears that animals with *PRNP* substitutions associated with reduced CWD susceptibility have a similar reproductive ability (DeVivo et al. 2017, Haley et al. 2020) and body condition (Haley et al. 2020) than those without them. Whether fitness traits are directly associated with either the various *PRNP* variants or to some loci genetically linked to them, should be determined. The effects on later PrP^{Sc} shedding and longer incubation periods, together with the host lifespan, should be assessed when evaluating the advantages and disadvantages for the overall population. Questions would be if animals carrying the less susceptible alleles die before becoming infectious (Jewell et al. 2005) or if their longer survival signifies a longer PrP^{Sc} shedding time with consequences in transmission risk for other animals (Plummer et al. 2017).

Last but not least, some *PRNP* alleles regarded as less susceptible in cervids have been implicated in the emergence of novel strains (Duque Velásquez et al. 2015, Moore et al. 2020), shifting the *PRNP* susceptibility towards patterns in which animals normally non-susceptible could become infected (Duque Velásquez et al. 2015). In

this sense, Wadsworth et al. (2021) questioned the zoonotic potential of novel CWD strains in Europe by using transgenic mice overexpressing human PrP and found that these strains represent a low risk. Further passages and additional models are required to evaluate this transmission barrier. There is still no consensus on the mechanisms behind the origins of new strains, but it seems that prions are adapting to changing environments (Duque Velásquez et al. 2020). In this context, preserving *PRNP* diversity may be beneficial for the long-term management of CWD to avoid high proportions of a *PRNP* alleles that might turn susceptible to a different strain or might affect the overall animal fitness.

7 Conclusions

This thesis has explored various aspects of *PRNP* variability in Norwegians cervids in the context of novel CWD strains identified in Europe. CWD in Norwegian reindeer shares many features with manifestation of CWD in North America with sex, age and *PRNP* genotype as risk factors for development of the disease. In Norwegian reindeer, case animals were not closely related although all affected animals originated from the same wild subpopulation. This situation is compatible with a horizontal mode of transmission. There was a clear male-biased risk of infection, for which the likelihood of infection increased with age. By controlling for sex and age, a genetic association between *PRNP* genotype *226Q*/266Q and *226Q*/*deletion_226Q* and the risk of testing CWD PrP^{Sc} positive was established, suggesting *226Q* to be a susceptible allele for the Norwegian reindeer strain.

CWD, as occurring in reindeer, is consistent with a contagious form of the disease, which leads to a potential transmission risk to other cervid species in Norway and other European countries. For instance, allele *226Q* is in fact found in four cervid species in Great Britain, including red deer, suggesting their potential susceptibility to CWD.

The frequencies of *PRNP* alleles diverge geographically as becomes evident in red deer, one of the most abundant wild deer species in Europe.

Spatial heterogeneity in *PRNP* was present among reindeer, red deer, and moose in Norway. Roe deer was monomorphic, but all species encoded allele *226Q*. The highest frequencies of allele *226Q* in red deer and moose were identified in genetically distinct subpopulations, while in reindeer the highest frequencies were predominantly identified in wild subpopulations. Various forces, including origin and adaptive processes might be acting on the *PRNP* differences between wild and semi-domestic reindeer.

The few cases of atypical CWD observed in Norwegian moose do not allow for robust conclusions, but there is a trend towards occurrence in homozygous animals. In conclusion, the *PRNP* variability identified in the present study suggests that Norwegian cervids are susceptible to CWD, although other conspecific features cannot be discounted.

8 Future perspectives

A recent case of contagious CWD in reindeer has been identified in Hardangervidda, a wild reindeer management area neighboring Nordfjella, where all previous cases were identified. This unfortunate scenario warns us about the possible occurrence of CWD into other populations. CWD control programmes and research into its recent emergence need to elucidate the factors that play a role in innate susceptibility, including *PRNP* variability. The applicability of *PRNP* variation as a management tool might be limited in part by the uncertain consequences changes in allele frequencies may have on the population. The novel CWD strains identified in Europe may limit the applicability of current knowledge based on genetic susceptibility reported from North America.

This thesis reports on *PRNP* allele variability among Norwegians cervids, which to date encompasses most known European CWD cases. A logical follow-up would be to study the significance of these *PRNP* alleles in relation to host physiology and CWD progression following exposure to novel strains. Studies including life history traits on animals with different *PRNP* genotypes might shed light on the consequences these may have on fitness. To further study the impact of these *PRNP* genotypes on the novel CWD strains, it would be advantageous to use a model system that provides a cervid cellular background, over an extended study period. A more "personalized" system could also allow for comparison between different species encoding a similar genotype, for example *226Q*/*226Q*, to test for other genetic modifiers involved in disease onset. The promoter region of *PRNP* appears to be a suitable candidate for further characterization in Norwegian cervids, considering substitutions in this region have recently being associated with increasing CWD risk in white-tailed deer.

An intriguing finding in this thesis is the disparate *PRNP* variation in wild and semi-domestic reindeer. It has been discussed whether this difference is due to adaptive processes related to the domestication processes, with certain *PRNP* variants related to behavioral or visuospatial traits. Characterization of *PRNP* variability in other closely co-existing wild and domestic reindeer herds could further test whether the domestication process *per se* has influenced *PRNP* variation in reindeer.

9 Reference list

Abskharon, R., F. Wang, A. Wohlkonig, J. Ruan, S. Soror, G. Giachin, E. Pardon, W. Zou, G. Legname, J. Ma and J. Steyaert (2019). "Structural evidence for the critical role of the prion protein hydrophobic region in forming an infectious prion." PLOS Pathogens **15**(12): e1008139.

Ager, A. A., B. K. Johnson, J. W. Kern and J. G. Kie (2003). "Daily and Seasonal Movements and Habitat Use by Female Rocky Mountain Elk and Mule Deer." Journal of Mammalogy **84**(3): 1076-1088.

Aguzzi, A., C. Sigurdson and M. Heikenwaelder (2008). "Molecular Mechanisms of Prion Pathogenesis." Annual Review of Pathology: Mechanisms of Disease **3**(1): 11-40.

Andersen, R., I. Herfindel, B.-E. Sæther, J. D. C. Linnell, J. Oddén and O. Liberg (2004). "When range expansion rate is faster in marginal habitats." Oikos **107**(1): 210-214.

Anderson, C. A., P. Bosque, C. M. Filley, D. B. Arciniegas, B. K. Kleinschmidt-DeMasters, W. J. Pape and K. L. Tyler (2007). "Colorado Surveillance Program for Chronic Wasting Disease Transmission to Humans: Lessons From 2 Highly Suspicious but Negative Cases." Archives of Neurology **64**(3): 439-441.

Angers, R., J. Christiansen, A. V. Nalls, H.-E. Kang, N. Hunter, E. Hoover, C. K. Mathiason, M. Sheetz and G. C. Telling (2014). "Structural effects of PrP polymorphisms on intra- and interspecies prion transmission." Proceedings of the National Academy of Sciences **111**(30): 11169-11174.

Angers, R. C., S. R. Browning, T. S. Seward, C. J. Sigurdson, M. W. Miller, E. A. Hoover and G. C. Telling (2006). "Prions in Skeletal Muscles of Deer with Chronic Wasting Disease." Science **311**(5764): 1117-1117.

Angers, R. C., T. S. Seward, D. Napier, M. Green, E. Hoover, T. Spraker, K. O'Rourke, A. Balachandran and G. C. Telling (2009). "Chronic wasting disease prions in elk antler velvet." Emerg Infect Dis **15**(5): 696-703.

Arifin, M. I., A. Staskevicius, S. Y. Shim, Y. H. Huang, H. Fenton, P. D. McLoughlin, G. Mitchell, C. I. Cullingham and S. Gilch (2020). "Large-scale prion protein genotyping in Canadian caribou populations and potential impact on chronic wasting disease susceptibility." Molecular Ecology **00**: 1-11.

Baeten, L. A., B. E. Powers, J. E. Jewell, T. R. Spraker and M. W. Miller (2007). "A Natural Case of Chronic Wasting Disease in a Free-ranging Moose (*Alces alces shirasi*)." Journal of Wildlife Diseases **43**(2): 309-314.

Balachandran, A., N. P. Harrington, J. Algire, A. Soutyrine, T. R. Spraker, M. Jeffrey, L. González and K. I. O'Rourke (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." The Canadian veterinary journal = La revue veterinaire canadienne **51**(2): 169-178.
Balding, D. J. (2006). "A tutorial on statistical methods for population association studies." Nature Reviews Genetics **7**: 781–791.
Basler, K., B. Oesch, M. Scott, D. Westaway, M. Wälchli, D. F. Groth, M. P. McKinley, S. B. Prusiner and C. Weissmann (1986). "Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene." Cell **46**(3): 417-428.
Baylis, M., F. Houston, W. Goldmann, N. Hunter and A. R. McLean (2000). "The signature of scrapie: differences in the PrP genotype profile of scrapie-affected and scrapie-free UK sheep flocks." Proceedings of the Royal Society of London. Series B: Biological Sciences **267**(1457): 2029-2035.
Baylis, M. and K. M. McIntyre (2004). "Scrapie control under new strain." Nature **432**(7019): 810-811.
Belay, E. D., P. Gambetti, L. B. Schonberger, P. Parchi, D. R. Lyon, S. Capellari, J. H. McQuiston, K. Bradley, G. Dowdle, J. M. Crutcher and C. R. Nichols (2001). "Creutzfeldt-Jakob Disease in Unusually Young Patients Who Consumed Venison." Archives of Neurology **58**(10): 1673-1678.
Benestad, S. L., G. Mitchell, M. Simmons, B. Ytrehus and T. Vikøren (2016). "First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer." Veterinary Research **47**(1): 88.
Benestad, S. L., P. Sarradin, B. Thu, J. Schönheit, M. A. Tranulis and B. Bratberg (2003). "Cases of scrapie with unusual features in Norway and designation of a new type, Nor98." Veterinary Record **153**(7): 202-208.
Benestad, S. L. and G. C. Telling (2018). Chapter 8 - Chronic wasting disease: an evolving prion disease of cervids. Handbook of Clinical Neurology. M. Pocchiari and J. Manson, Elsevier. **153:** 135-151.
Bian, J., J. R. Christiansen, J. A. Moreno, S. J. Kane, V. Khaychuk, J. Gallegos, S. Kim and G. C. Telling (2019). "Primary structural differences at residue 226 of deer and elk PrP dictate selection of distinct CWD prion strains in gene-targeted mice." Proceedings of the National Academy of Sciences **116**(25): 12478-12487.
Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S. L. Sunden, G. A. Hawkins, S. S. Toldo, R. Fries, M. D. Grosz and J. Yoo (1994). "A genetic linkage map for cattle." Genetics **136**(2): 619-639.
Bistaffa, E., T. T. Vuong, F. A. Cazzaniga, L. Tran, G. Salzano, G. Legname, G. Giaccone, S. L. Benestad and F. Moda (2019). "Use of different RT-QuIC substrates for detecting CWD prions in the brain of Norwegian cervids." Scientific Reports **9**: 18595.

Bolton, D., M. McKinley and S. Prusiner (1982). "Identification of a protein that purifies with the scrapie prion." Science **218**(4579): 1309-1311.
Brandner, S., S. Isenmann, A. Raeber, M. Fischer, A. Sailer, Y. Kobayashi, S. Marino, C. Weissmann and A. Aguzzi (1996). "Normal host prion protein necessary for scrapie-induced neurotoxicity." Nature **379**(6563): 339-343.
Brandt, A. L., M. L. Green, Y. Ishida, A. L. Roca, J. Novakofski and N. E. Mateus-Pinilla (2018). "Influence of the geographic distribution of prion protein gene sequence variation on patterns of chronic wasting disease spread in white-tailed deer (*Odocoileus virginianus*)." Prion **12**(3-4): 204-215.
Brandt, A. L., A. C. Kelly, M. L. Green, P. Shelton, J. Novakofski and N. E. Mateus-Pinilla (2015). "Prion protein gene sequence and chronic wasting disease susceptibility in white-tailed deer (*Odocoileus virginianus*)." Prion **9**(6): 449-462.
Brayton, K. A., K. I. O'Rourke, A. K. Lyda, M. W. Miller and D. P. Knowles (2004). "A processed pseudogene contributes to apparent mule deer prion gene heterogeneity." Gene **326**: 167-173.
Bruce, M. E., R. G. Will, J. W. Ironside, I. McConnell, D. Drummond, A. Suttie, L. McCardle, A. Chree, J. Hope, C. Birkett, S. Cousens, H. Fraser and C. J. Bostock (1997). "Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent." Nature **389**(6650): 498-501.
Bruford, E. A., B. Braschi, P. Denny, T. E. M. Jones, R. L. Seal and S. Tweedie (2020). "Guidelines for human gene nomenclature." Nature Genetics **52**(8): 754-758.
Buchanan, F. C. and A. M. Crawford (1993). "Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci." Animal Genetics **24**(2): 145-145.
Büeler, H., A. Aguzzi, A. Sailer, R. A. Greiner, P. Autenried, M. Aguet and C. Weissmann (1993). "Mice devoid of PrP are resistant to scrapie." Cell **73**(7): 1339-1347.
Calzolai, L., D. A. Lysek, D. R. Pérez, P. Güntert and K. Wüthrich (2005). "Prion protein NMR structures of chickens, turtles, and frogs." Proceedings of the National Academy of Sciences of the United States of America **102**(3): 651-655.
Carlson, G. A., D. Westaway, S. J. DeArmond, M. Peterson-Torchia and S. B. Prusiner (1989). "Primary structure of prion protein may modify scrapie isolate properties." Proceedings of the National Academy of Sciences **86**(19): 7475-7479.
Casalone, C., G. Zanusso, P. Acutis, S. Ferrari, L. Capucci, F. Tagliavini, S. Monaco and M. Caramelli (2004). "Identification of a second bovine amyloidotic spongiform encephalopathy: Molecular similarities with sporadic Creutzfeldt-Jakob disease." Proceedings of the National Academy of Sciences **101**(9): 3065-3070.

Cassard, H., J.-M. Torres, C. Lacroux, J.-Y. Douet, S. L. Benestad, F. Lantier, S. Lugan, I. Lantier, P. Costes, N. Aron, F. Reine, L. Herzog, J.-C. Espinosa, V. Beringue and O. Andréoletti (2014). "Evidence for zoonotic potential of ovine scrapie prions." Nature Communications **5**(1): 5821.
Chafin, T. K., M. R. Douglas, B. T. Martin, Z. D. Zbinden, C. R. Middaugh, J. R. Ballard, M. C. Gray, D. White and M. E. Douglas (2020). "Age structuring and spatial heterogeneity in prion protein gene (*PRNP*) polymorphism in white-tailed deer." Prion **14**(1): 238-248.
Cheng, Y. C., M. Musiani, M. Cavedon and S. Gilch (2017). "High prevalence of prion protein genotype associated with resistance to chronic wasting disease in one Alberta woodland caribou population." Prion **11**(2): 136-142.
Chesebro, B., R. Race, K. Wehrly, J. Nishio, M. Bloom, D. Lechner, S. Bergstrom, K. Robbins, L. Mayer, J. M. Keith, C. Garon and A. Haase (1985). "Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain." Nature **315**(6017): 331-333.
Christen, B., S. Hornemann, F. F. Damberger and K. Wüthrich (2009). "Prion Protein NMR Structure from Tammar Wallaby (*Macropus eugenii*) Shows that the β2–α2 Loop Is Modulated by Long-Range Sequence Effects." Journal of Molecular Biology **389**(5): 833-845.
Christen, B., K. Wüthrich and S. Hornemann (2008). "Putative prion protein from Fugu (*Takifugu rubripes*)." The FEBS Journal **275**(2): 263-270.
Colby, D. W. and S. B. Prusiner (2011). "Prions." Cold Spring Harbor Perspectives in Biology **3**: a006833.
Comoy, E. E., J. Mikol, S. Luccantoni-Freire, E. Correia, N. Lescoutra-Etchegaray, V. Durand, C. Dehen, O. Andreoletti, C. Casalone, J. A. Richt, J. J. Greenlee, T. Baron, S. L. Benestad, P. Brown and J.-P. Deslys (2015). "Transmission of scrapie prions to primate after an extended silent incubation period." Scientific Reports **5**(1): 11573.
Cuillé, J., Che and P.-L. lle (1936). "*La maladie dite* tremblante du mouton *est-elle inoculable*?" Comptes rendus hebdomadaires des séances de l'Académie des sciences **203**: 1552-1554.
Cullingham, C. I., E. H. Merrill, M. J. Pybus, T. K. Bollinger, G. A. Wilson and D. W. Coltman (2010). "Broad and fine-scale genetic analysis of white-tailed deer populations: estimating the relative risk of chronic wasting disease spread." Evol Appl **4**(1): 116-131.
Cullingham, C. I., S. M. Nakada, E. H. Merrill, T. K. Bollinger, M. J. Pybus and D. W. Coltman (2011). "Multiscale population genetic analysis of mule deer (*Odocoileus hemionus hemionus*) in western Canada sheds new light on the spread of chronic wasting disease." Canadian Journal of Zoology **89**(2): 134-147.
Cullingham, C. I., R. M. Peery, A. Dao, D. I. McKenzie and D. W. Coltman (2020). "Predicting the spread-risk potential of chronic wasting disease to sympatric ungulate species." Prion **14**(1): 56-66.

Curcio, L., C. Sebastiani, P. Di Lorenzo, E. Lasagna and M. Biagetti (2016). "Review: A review on classical and atypical scrapie in caprine: Prion protein gene polymorphisms and their role in the disease." Animal **10**(10): 1585-1593.
Davenport, K. A., J. R. Christiansen, J. Bian, M. Young, J. Gallegos, S. Kim, A. Balachandran, C. K. Mathiason, E. A. Hoover and G. C. Telling (2018). "Comparative analysis of prions in nervous and lymphoid tissues of chronic wasting disease-infected cervids." Journal of General Virology **99**(5).
den Dunnen, J. T., R. Dalgleish, D. R. Maglott, R. K. Hart, M. S. Greenblatt, J. McGowan-Jordan, A. F. Roux, T. Smith, S. E. Antonarakis and P. E. Taschner (2016). "HGVS Recommendations for the Description of Sequence Variants: 2016 Update." Human Mutation **37**: 564-569.
Denny, G. O. and W. D. Hueston (1997). "Epidemiology of bovine spongiform encephalopathy in Northern Ireland 1988 to 1995." Veterinary Record **140**(12): 302-306.
DeVivo, M. T., D. R. Edmunds, M. J. Kauffman, B. A. Schumaker, J. Binfet, T. J. Kreeger, B. J. Richards, H. M. Schätzl and T. E. Cornish (2017). "Endemic chronic wasting disease causes mule deer population decline in Wyoming." PLOS ONE **12**(10): e0186512.
Donne, D. G., J. H. Viles, D. Groth, I. Mehlhorn, T. L. James, F. E. Cohen, S. B. Prusiner, P. E. Wright and H. J. Dyson (1997). "Structure of the recombinant full-length hamster prion protein PrP(29–231): The N terminus is highly flexible." Proceedings of the National Academy of Sciences **94**(25): 13452-13457.
Drew, E. D. and R. W. Janes (2019). "2StrucCompare: a webserver for visualizing small but noteworthy differences between protein tertiary structures through interrogation of the secondary structure content." Nucleic Acids Research **47**(W1): W477-W481.
Duque Velásquez, C., C. Kim, T. Haldiman, C. Kim, A. Herbst, J. M. Aiken, J. G. Safar and D. McKenzie (2020). "Chronic wasting disease (CWD) prion strains evolve via adaptive diversification of conformers in hosts expressing prion protein polymorphisms." Journal of Biological Chemistry **295**(15): 4985–5001.
Duque Velásquez, C., C. Kim, A. Herbst, N. Daude, M. C. Garza, H. Wille, J. Aiken and D. McKenzie (2015). "Deer Prion Proteins Modulate the Emergence and Adaptation of Chronic Wasting Disease Strains." Journal of Virology **89**(24): 12362-12373.
Ellegren, H. (2004). "Microsatellites: simple sequences with complex evolution." Nature Reviews Genetics **5**(6): 435-445.

Finnish Food Authority - *Ruokavirasto*. (2020, 2020-11-13). "TSE found in a moose killed in Laukaa." Seinäjoki, Finland. Available at: https://www.ruokavirasto.fi/en/farmers/animal-husbandry/animal-health-and-diseases/animal-diseases/news/tse-found-in-a-moose-killed-in-laukaa/ (accessed on April 14th, 2021).
Flagstad, Ø. and K. H. Røed (2003). "REFUGIAL ORIGINS OF REINDEER (*RANGIFER TARANDUS L.*) INFERRED FROM MITOCHONDRIAL DNA SEQUENCES." Evolution **57**(3): 658-670.
Fox, K. A., J. E. Jewell, E. S. Williams and M. W. Miller (2006). "Patterns of PrP^{CWD} accumulation during the course of chronic wasting disease infection in orally inoculated mule deer (*Odocoileus hemionus*)." Journal of General Virology **87**(11): 3451-3461.
Frishman, D. and P. Argos (1995). "Knowledge-based protein secondary structure assignment." Proteins: Structure, Function, and Bioinformatics **23**(4): 566-579.
Gabriel, J. M., B. Oesch, H. Kretzschmar, M. Scott and S. B. Prusiner (1992). "Molecular cloning of a candidate chicken prion protein." Proceedings of the National Academy of Sciences **89**(19): 9097-9101.
Gajdusek, D. C., C. J. Gibbs and M. Alpers (1966). "Experimental Transmission of a Kuru-like Syndrome to Chimpanzees." Nature **209**(5025): 794-796.
Gambetti, P., Q. Kong, W. Zou, P. Parchi and S. G. Chen (2003). "Sporadic and familial CJD: classification and characterisation." British Medical Bulletin **66**(1): 213-239.
Gibbs, C. J., D. C. Gajdusek, D. M. Asher, M. P. Alpers, E. Beck, P. M. Daniel and W. B. Matthews (1968). "Creutzfeldt-Jakob Disease (Spongiform Encephalopathy): Transmission to the Chimpanzee." Science **161**(3839): 388-389.
Gossert, A. D., S. Bonjour, D. A. Lysek, F. Fiorito and K. Wüthrich (2005). "Prion protein NMR structures of elk and of mouse/elk hybrids." Proceedings of the National Academy of Sciences of the United States of America **102**(3): 646-650.
Grear, D. A., M. D. Samuel, K. T. Scribner, B. V. Weckworth and J. A. Langenberg (2010). "Influence of genetic relatedness and spatial proximity on chronic wasting disease infection among female white-tailed deer." Journal of Applied Ecology **47**(3): 532-540.
Greenlee, J. J., E. M. Nicholson, J. D. Smith, R. A. Kunkle and A. N. Hamir (2012). "Susceptibility of cattle to the agent of chronic wasting disease from elk after intracranial inoculation." Journal of Veterinary Diagnostic Investigation **24**(6): 1087-1093.
Grendstad (e.f.), E. E. and S. I. Ormsettrø (2017). Tiltak mot CWD/skrantesjuke i Nordfjella villreinområde. 16/474-. D. K. L.-o. matdepartementet. Oslo, Regjeringen.no.

Guichoux, E., L. Lagache, S. Wagner, P. Chaumeil, P. Léger, O. Lepais, C. Lepoittevin, T. Malausa, E. Revardel, F. Salin and R. J. Petit (2011). "Current trends in microsatellite genotyping." Molecular Ecology Resources **11**(4): 591-611.
Guo, S. W. and E. A. Thompson (1992). "Performing the exact test of Hardy-Weinberg proportion for multiple alleles." Biometrics **48**(2): 361-372.
Hadlow, W. J. (1959). "SCRAPIE AND KURU." The Lancet **274**(7097): 289-290.
Haigh, C. L., J. A. Wright and D. R. Brown (2007). "Regulation of Prion Protein Expression by Noncoding Regions of the *Prnp* Gene." Journal of Molecular Biology **368**(4): 915-927.
Haley, N. J., D. M. Henderson, R. Donner, S. Wyckoff, K. Merrett, J. Tennant, E. A. Hoover, D. Love, E. Kline, A. D. Lehmkuhl and B. V. Thomsen (2020). "Management of chronic wasting disease in ranched elk: conclusions from a longitudinal three-year study." Prion **14**(1): 76-87.
Haley, N. J., K. Merrett, A. Buros Stein, D. Simpson, A. Carlson, G. Mitchell, A. Staskevicius, T. Nichols, A. D. Lehmkuhl and B. V. Thomsen (2019). "Estimating relative CWD susceptibility and disease progression in farmed white-tailed deer with rare PRNP alleles." PLOS ONE **14**(12): e0224342.
Haley, N. J., R. Rielinger, K. A. Davenport, K. O'Rourke, G. Mitchell and J. A. Richt (2017). "Estimating chronic wasting disease susceptibility in cervids using real-time quaking-induced conversion." J Gen Virol **98**(11): 2882-2892.
Haley, N. J., C. Siepker, J. J. Greenlee and J. A. Richt (2016). "Limited amplification of chronic wasting disease prions in the peripheral tissues of intracerebrally inoculated cattle." Journal of General Virology **97**(7): 1720-1724.
Haley, N. J., C. Siepker, L. L. Hoon-Hanks, G. Mitchell, W. D. Walter, M. Manca, R. J. Monello, J. G. Powers, M. A. Wild, E. A. Hoover, B. Caughey and J. A. Richt (2016). "Seeded Amplification of Chronic Wasting Disease Prions in Nasal Brushings and Recto-anal Mucosa-Associated Lymphoid Tissues from Elk by Real-Time Quaking-Induced Conversion." Journal of Clinical Microbiology **54**(4): 1117-1126.
Haley, N. J., C. Siepker, W. D. Walter, B. V. Thomsen, J. J. Greenlee, A. D. Lehmkuhl and J. A. Richt (2016). "Antemortem Detection of Chronic Wasting Disease Prions in Nasal Brush Collections and Rectal Biopsy Specimens from White-Tailed Deer by Real-Time Quaking-Induced Conversion." Journal of Clinical Microbiology **54**(4): 1108-1116.
Hamir, A. N., T. Gidlewski, T. R. Spraker, J. M. Miller, L. Creekmore, M. Crocheck, T. Cline and K. I. O'Rourke (2006). "Preliminary Observations of Genetic Susceptibility of elk (*Cervus Elaphus Nelsoni*) to Chronic Wasting Disease by Experimental Oral Inoculation." Journal of Veterinary Diagnostic Investigation **18**(1): 110-114.

Hamir, A. N., J. J. Greenlee, E. M. Nicholson, R. A. Kunkle, J. A. Richt, J. M. Miller and M. Hall (2011). "Experimental transmission of chronic wasting disease (CWD) from elk and white-tailed deer to fallow deer by intracerebral route: Final report." Canadian Journal of Veterinary Research **75**(2): 152-156.
Hamir, A. N., R. A. Kunkle, R. C. Cutlip, J. M. Miller, K. I. O'Rourke, E. S. Williams, M. W. Miller, M. J. Stack, M. J. Chaplin and J. A. Richt (2005). "Experimental Transmission of Chronic Wasting Disease Agent from Mule Deer to Cattle by the Intracerebral Route." Journal of Veterinary Diagnostic Investigation **17**(3): 276-281.
Hamir, A. N., R. A. Kunkle, R. C. Cutlip, J. M. Miller, E. S. Williams and J. A. Richt (2006). "Transmission of Chronic Wasting Disease of Mule Deer to Suffolk Sheep following Intracerebral Inoculation." Journal of Veterinary Diagnostic Investigation **18**(6): 558-565.
Hamir, A. N., J. M. Miller, R. A. Kunkle, S. M. Hall and J. A. Richt (2007). "Susceptibility of Cattle to First-passage Intracerebral Inoculation with Chronic Wasting Disease Agent from White-tailed Deer." Veterinary Pathology **44**(4): 487-493.
Happ, G. M., H. J. Huson, K. B. Beckmen and L. J. Kennedy (2007). "PRION PROTEIN GENES IN CARIBOU FROM ALASKA." Journal of Wildlife Diseases **43**(2): 224-228.
Harrison, C. F., V. A. Lawson, B. M. Coleman, Y.-S. Kim, C. L. Masters, R. Cappai, K. J. Barnham and A. F. Hill (2010). "Conservation of a Glycine-rich Region in the Prion Protein Is Required for Uptake of Prion Infectivity." Journal of Biological Chemistry **285**(26): 20213-20223.
Harrison, P. M., A. Khachane and M. Kumar (2010). "Genomic assessment of the evolution of the prion protein gene family in vertebrates." Genomics **95**(5): 268-277.
Heaton, M. P., K. A. Leymaster, B. A. Freking, D. A. Hawk, T. P. L. Smith, J. W. Keele, W. M. Snelling, J. M. Fox, C. G. Chitko-McKown and W. W. J. M. G. Laegreid (2003). "Prion gene sequence variation within diverse groups of U.S. sheep, beef cattle, and deer." Mamm Genome **14**(11): 765-777.
Hill, A. F., M. Desbruslais, S. Joiner, K. C. L. Sidle, I. Gowland, J. Collinge, L. J. Doey and P. Lantos (1997). "The same prion strain causes vCJD and BSE." Nature **389**(6650): 448-450.
Hoover, C. E., K. A. Davenport, D. M. Henderson, N. D. Denkers, C. K. Mathiason, C. Soto, M. D. Zabel and E. A. Hoover (2017). "Pathways of Prion Spread during Early Chronic Wasting Disease in Deer." Journal of Virology **91**(10): e00077-00017.
Hosszu, L. L. P., R. Conners, D. Sangar, M. Batchelor, E. B. Sawyer, S. Fisher, M. J. Cliff, A. M. Hounslow, K. McAuley, R. Leo Brady, G. S. Jackson, J. Bieschke, J. P. Waltho and J. Collinge (2020). "Structural effects of the highly protective V127 polymorphism on human prion protein." Communications Biology **3**(1): 402.

Houlihan, L. M., S. E. Harris, M. Luciano, A. J. Gow, J. M. Starr, P. M. Visscher and I. J. Deary (2009). "Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936." Genes Brain Behav **8**(2): 238-247.
Hsiao, K., H. F. Baker, T. J. Crow, M. Poulter, F. Owen, J. D. Terwilliger, D. Westaway, J. Ott and S. B. Prusiner (1989). "Linkage of a prion protein missense variant to Gerstmann–Sträussler syndrome." Nature **338**(6213): 342-345.
Hundertmark, K. J., G. F. Shields, I. G. Udina, R. T. Bowyer, A. A. Danilkin and C. C. Schwartz (2002). "Mitochondrial Phylogeography of Moose (*Alces alces*): Late Pleistocene Divergence and Population Expansion." Molecular Phylogenetics and Evolution **22**(3): 375-387.
Huson, H. J. and G. M. Happ (2006). "Polymorphisms of the prion protein gene (*PRNP*) in Alaskan moose (*Alces alces gigas*)." Animal Genetics **37**(4): 425-426.
Haanes, H., K. H. Røed, Ø. Flagstad and O. Rosef (2010). "Genetic structure in an expanding cervid population after population reduction." Conservation Genetics **11**(1): 11-20.
Haanes, H., K. H. Røed, E. J. Solberg, I. Herfindal and B.-E. Sæther (2011). "Genetic discontinuities in a continuously distributed and highly mobile ungulate, the Norwegian moose." Conservation Genetics **12**(5): 1131.
Ishida, Y., T. Tian, A. L. Brandt, A. C. Kelly, P. Shelton, A. L. Roca, J. Novakofski and N. E. Mateus-Pinilla (2020). "Association of chronic wasting disease susceptibility with prion protein variation in white-tailed deer (*Odocoileus virginianus*)." Prion **14**(1): 214-225.
Jewell, J. E., M. M. Conner, L. L. Wolfe, M. W. Miller and E. S. Williams (2005). "Low frequency of PrP genotype 225SF among free-ranging mule deer (*Odocoileus hemionus*) with chronic wasting disease." Journal of General Virology **86**(8): 2127-2134.
Johnson, C., J. Johnson, J. P. Vanderloo, D. Keane, J. M. Aiken and D. McKenzie (2006). "Prion protein polymorphisms in white-tailed deer influence susceptibility to chronic wasting disease." Journal of General Virology **87**(7): 2109-2114.
Johnson, C. J., A. Herbst, C. Duque-Velasquez, J. P. Vanderloo, P. Bochsler, R. Chappell and D. McKenzie (2011). "Prion Protein Polymorphisms Affect Chronic Wasting Disease Progression." PLOS ONE **6**(3): e17450.
Joly, D. O., M. D. Samuel, J. A. Langenberg, J. A. Blanchong, C. A. Batha, R. E. Rolley, D. P. Keane and C. A. Ribic (2006). "SPATIAL EPIDEMIOLOGY OF CHRONIC WASTING DISEASE IN WISCONSIN WHITE-TAILED DEER." Journal of Wildlife Diseases **42**(3): 578-588.
Jones, K. C., K. F. Levine and J. D. Banks (2000). "DNA-based genetic markers in black-tailed nad mule deer for forensic applications." California Fish and Game **86**(2): 115-126.

Kahn, S., C. Dubé, L. Bates and A. Balachandran (2004). "Chronic wasting disease in Canada: Part 1." Can Vet J **45**(5): 397-404.
Kallunki Nyström, J. (2019). An investigation of moose calves from females with Moose Wasting Syndrome (*Alces alces L.*). Degree Project in Veterinary Medicine.
Kaluz, S., M. Kaluzova and A. P. F. Flint (1997). "Sequencing analysis of prion genes from red deer and camel." Gene **199**(1): 283-286.
Keane, D. P., D. J. Barr, P. N. Bochsler, S. M. Hall, T. Gidlewski, K. I. O'Rourke, T. R. Spraker and M. D. Samuel (2008). "Chronic Wasting Disease in a Wisconsin White-Tailed Deer Farm." J Vet Diagn Invest **20**(5): 698-703.
Kelly, A. C., N. E. Mateus-Pinilla, J. Diffendorfer, E. Jewell, M. O. Ruiz, J. Killefer, P. Shelton, T. Beissel and J. Novakofski (2008). "Prion sequence polymorphisms and chronic wasting disease resistance in Illinois white-tailed deer (*Odocoileus virginianus*)." Prion **2**(1): 28-36.
Kelly, A. C., N. E. Mateus-Pinilla, M. Douglas, M. Douglas, W. Brown, M. O. Ruiz, J. Killefer, P. Shelton, T. Beissel and J. Novakofski (2010). "Utilizing disease surveillance to examine gene flow and dispersal in white-tailed deer." Journal of Applied Ecology **47**(6): 1189-1198.
Kholodova, M. V., A. I. Baranova, I. A. Mizin, D. V. Panchenko, T. M. Romanenko and A. N. Korolev (2019). "A Genetic Predisposition to Chronic Wasting Disease in the Reindeer *Rangifer tarandus* in the Northern European Part of Russia." Biology Bulletin **46**(6): 555-561.
Kim, T.-Y., H.-J. Shon, Y.-S. Joo, U.-K. Mun, K.-S. Kang and Y.-S. Lee (2005). "Additional Cases of Chronic Wasting Disease in Imported Deer in Korea." Journal of Veterinary Medical Science **67**(8): 753-759.
Kim, Y., J. Lee and C. Lee (2008). "*In silico* Comparative Analysis of DNA and Amino Acid Sequences for Prion Protein Gene." Transboundary and Emerging Diseases **55**(2): 105-114.
Kitsios, G. D. and E. Zintzaras (2009). "Genome-wide association studies: hypothesis-"free" or "engaged"?" Translational Research **154**(4): 161-164.
Klatzo, I., D. C. Gajdusek and V. Zigas (1959). "Pathology of Kuru." Lab Invest **8**(4): 799-847.
Kreeger, T. J., D. L. Montgomery, J. E. Jewell, W. Schultz and E. S. Williams (2006). "Oral Transmission of Chronic Wasting Disease in Captive Shira's Moose." Journal of Wildlife Diseases **42**(3): 640-645.
Krojerová-Prokešová, J., M. Barančeková and P. Koubek (2015). "Admixture of Eastern and Western European Red Deer Lineages as a Result of Postglacial Recolonization of the Czech Republic (Central Europe)." Journal of Heredity **106**(4): 375-385.
Kvie, K. S., J. Heggenes, B.-J. Bårdsen and K. H. Røed (2019). "Recent large-scale landscape changes, genetic drift and reintroductions characterize the genetic structure of Norwegian wild reindeer." Conservation Genetics **20**: 1405–1419.

Kvie, K. S., J. Heggenes and K. H. Røed (2016). "Merging and comparing three mitochondrial markers for phylogenetic studies of Eurasian reindeer (*Rangifer tarandus*)." Ecology and Evolution **6**(13): 4347-4358.
Lasmézas, C. I., E. Comoy, S. Hawkins, C. Herzog, F. Mouthon, T. Konold, F. Auvré, E. Correia, N. Lescoutra-Etchegaray, N. Salès, G. Wells, P. Brown and J.-P. Deslys (2005). "Risk of oral infection with bovine spongiform encephalopathy agent in primates." The Lancet **365**(9461): 781-783.
Latarjet, R., B. Muel, D. A. Haig, M. C. Clarke and T. Alper (1970). "Inactivation of the Scrapie Agent by Near Monochromatic Ultraviolet Light." Nature **227**(5265): 1341-1343.
Le Pichon, C. E., M. T. Valley, M. Polymenidou, A. T. Chesler, B. T. Sagdullaev, A. Aguzzi and S. Firestein (2009). "Olfactory behavior and physiology are disrupted in prion protein knockout mice." Nature Neuroscience **12**(1): 60-69.
Lee, H.-S., P. Brown, L. Cervenáková, R. M. Garruto, M. P. Alpers, D. C. Gajdusek and L. G. Goldfarb (2001). "Increased Susceptibility to Kuru of Carriers of the *PRNP* 129 Methionine/Methionine Genotype." The Journal of Infectious Diseases **183**(2): 192-196.
Lee, I. Y., D. Westaway, A. F. A. Smit, K. Wang, J. Seto, L. Chen, C. Acharya, M. Ankener, D. Baskin, C. Cooper, H. Yao, S. B. Prusiner and L. E. Hood (1998). "Complete Genomic Sequence and Analysis of the Prion Protein Gene Region from Three Mammalian Species." Genome Research **8**(10): 1022-1037.
Lee, Y.-H., H.-J. Sohn, M.-J. Kim, H.-J. Kim, W.-Y. Lee, E.-I. Yun, D.-S. Tark, I.-S. Cho and A. Balachandran (2013). "Strain Characterization of the Korean CWD Cases in 2001 and 2004." Journal of Veterinary Medical Science **75**(1): 95-98.
Lewis, C. M. (2002). "Genetic association studies: Design, analysis and interpretation." Briefings in Bioinformatics **3**(2): 146-153.
Licinio, J. (2010). "Messages from hypothesis-driven genotyping: the case of schizoaffective disorder, bipolar type." Molecular Psychiatry **15**(2): 113-114.
Linnell, J. D. C., B. Cretois, E. B. Nilsen, C. M. Rolandsen, E. J. Solberg, V. Veiberg, P. Kaczensky, B. Van Moorter, M. Panzacchi, G. R. Rauset and B. Kaltenborn (2020). "The challenges and opportunities of coexisting with wild ungulates in the human-dominated landscapes of Europe's Anthropocene." Biological Conservation **244**: 108500.
Linsenmeier, L., H. C. Altmeppen, S. Wetzel, B. Mohammadi, P. Saftig and M. Glatzel (2017). "Diverse functions of the prion protein – Does proteolytic processing hold the key?" Biochimica et Biophysica Acta (BBA) - Molecular Cell Research **1864**(11, Part B): 2128-2137.

Little, J., J. P. T. Higgins, J. P. A. Ioannidis, D. Moher, F. Gagnon, E. von Elm, M. J. Khoury, B. Cohen, G. Davey-Smith, J. Grimshaw, P. Scheet, M. Gwinn, R. E. Williamson, G. Y. Zou, K. Hutchings, C. Y. Johnson, V. Tait, M. Wiens, J. Golding, C. van Duijn, J. McLaughlin, A. Paterson, G. Wells, I. Fortier, M. Freedman, M. Zecevic, R. King, C. Infante-Rivard, Stewart, Alex and N. Birkett (2009). "STrengthening the REporting of Genetic Association Studies (STREGA)—an extension of the STROBE statement." Genetic Epidemiology **33**(7): 581-598.
Lukic, A. and S. Mead (2011). "Genome wide association studies and prion disease." Prion **5**(3): 154-160.
Lynch, M. and K. Ritland (1999). "Estimation of Pairwise Relatedness With Molecular Markers." Genetics **152**(4): 1753-1766.
Makrinou, E., J. Collinge and M. Antoniou (2002). "Genomic characterization of the human prion protein (PrP) gene locus." Mammalian Genome **13**(12): 696-703.
Mallucci, G., A. Dickinson, J. Linehan, P.-C. Klöhn, S. Brandner and J. Collinge (2003). "Depleting Neuronal PrP in Prion Infection Prevents Disease and Reverses Spongiosis." Science **302**(5646): 871-874.
Manjerovic, M. B., M. L. Green, N. Mateus-Pinilla and J. Novakofski (2014). "The importance of localized culling in stabilizing chronic wasting disease prevalence in white-tailed deer populations." Preventive Veterinary Medicine **113**(1): 139-145.
Martin, R., P.-F. Gallet, D. Rocha and D. Petit (2009). "Polymorphism of the Prion Protein in Mammals: A Phylogenetic Approach." Recent Patents on DNA & Gene Sequences **3**(1): 63-71.
Martin, S., M. Jeffrey, L. González, S. Sisó, H. W. Reid, P. Steele, M. P. Dagleish, M. J. Stack, M. J. Chaplin and A. Balachandran (2009). "Immunohistochemical and biochemical characteristics of BSE and CWD in experimentally infected European red deer (*Cervus elaphus elaphus*)." BMC Veterinary Research **5**(1): 26.
Martucci, F., P. Acutis, M. Mazza, S. Nodari, S. Colussi, C. Corona, S. Barocci, A. Gabrielli, M. Caramelli, C. Casalone and G. Moroncini (2009). "Detection of typical and atypical bovine spongiform encephalopathy and scrapie prion strains by prion protein motif-grafted antibodies." Journal of General Virology **90**(4): 1048-1053.
Mathiason, C. K., S. A. Hays, J. Powers, J. Hayes-Klug, J. Langenberg, S. J. Dahmes, D. A. Osborn, K. V. Miller, R. J. Warren, G. L. Mason and E. A. Hoover (2009). "Infectious Prions in Pre-Clinical Deer and Transmission of Chronic Wasting Disease Solely by Environmental Exposure." PLOS ONE **4**(6): e5916.
McDonald, A. J., J. P. Dibble, E. G. B. Evans and G. L. Millhauser (2014). "A New Paradigm for Enzymatic Control of α-Cleavage and β-Cleavage of the Prion Protein." Journal of Biological Chemistry **289**(2): 803-813.
McGowan, J. P. (1922). "Scrapie in sheep." The Scottish Journal of Agriculture: 365-375.

Mead, S., J. Uphill, J. Beck, M. Poulter, T. Campbell, J. Lowe, G. Adamson, H. Hummerich, N. Klopp, I.-M. Rückert, H.-E. Wichmann, D. Azazi, V. Plagnol, W. H. Pako, J. Whitfield, M. P. Alpers, J. Whittaker, D. J. Balding, I. Zerr, H. Kretzschmar and J. Collinge (2011). "Genome-wide association study in multiple human prion diseases suggests genetic risk factors additional to *PRNP*." Human Molecular Genetics **21**(8): 1897-1906.
Miller, M. W., N. T. Hobbs and S. J. Tavener (2006). "DYNAMICS OF PRION DISEASE TRANSMISSION IN MULE DEER." Ecological Applications **16**(6): 2208-2214.
Miller, M. W. and M. A. Wild (2004). "Epidemiology of Chronic Wasting Disease in Captive White-Tailed and Mule Deer." Journal of Wildlife Diseases **40**(2): 320-327.
Miller, M. W., E. S. Williams, C. W. McCarty, T. R. Spraker, T. J. Kreeger, C. T. Larsen and E. T. Thorne (2000). "EPIZOOTIOLOGY OF CHRONIC WASTING DISEASE IN FREE-RANGING CERVIDS IN COLORADO AND WYOMING." Journal of Wildlife Diseases **36**(4): 676-690.
Miller, W. L. and W. D. Walter (2019). "Spatial heterogeneity of prion gene polymorphisms in an area recently infected by chronic wasting disease." Prion **13**(1): 65-76.
Mitchell, G. B., C. J. Sigurdson, K. I. O'Rourke, J. Algire, N. P. Harrington, I. Walther, T. R. Spraker and A. Balachandran (2012). "Experimental Oral Transmission of Chronic Wasting Disease to Reindeer (*Rangifer tarandus tarandus*)." PLOS ONE **7**(6): e39055.
Monello, R. J., N. L. Galloway, J. G. Powers, S. A. Madsen-Bouterse, W. H. Edwards, M. E. Wood, K. I. O'Rourke and M. A. Wild (2017). "Pathogen-mediated selection in free-ranging elk populations infected by chronic wasting disease." Proceedings of the National Academy of Sciences **114**(46): 12208-12212.
Moore, J., T. Tatum, S. Hwang, C. Vrentas, M. H. West Greenlee, Q. Kong, E. Nicholson and J. Greenlee (2020). "Novel Strain of the Chronic Wasting Disease Agent Isolated From Experimentally Inoculated Elk With LL132 Prion Protein." Scientific Reports **10**(1): 3148.
Moore, S. J., R. Kunkle, M. H. West Greenlee, E. Nicholson, J. Richt, A. Hamir, W. R. Waters and J. Greenlee (2016). "Horizontal Transmission of Chronic Wasting Disease in Reindeer." Emerging Infectious Disease journal **22**(12): 2142-2145.
Moum, T., I. Olsaker, P. Hopp, T. Moldal, M. Valheim, T. Moum and S. L. Benestad (2005). "Polymorphisms at codons 141 and 154 in the ovine prion protein gene are associated with scrapie Nor98 cases." Journal of General Virology **86**(1): 231-235.
Mysterud, A., S. L. Benestad, C. M. Rolandsen and J. Våge (2021). "Policy implications of an expanded chronic wasting disease universe." Journal of Applied Ecology **58**(2): 281-285.

Mysterud, A. and C. M. Rolandsen (2018). "A reindeer cull to prevent chronic wasting disease in Europe." Nature Ecology & Evolution **2**: 1343–1345.
Nalls, A. V., E. McNulty, J. Powers, D. M. Seelig, C. Hoover, N. J. Haley, J. Hayes-Klug, K. Anderson, P. Stewart, W. Goldmann, E. A. Hoover and C. K. Mathiason (2013). "Mother to Offspring Transmission of Chronic Wasting Disease in Reeves' Muntjac Deer." PLOS ONE **8**(8): e71844.
National Veterinary Institute - *Statens veterinärmedicinska anstalt*. (2020, 2020-09-24). "Map of Chronic Wasting Disease (CWD)." Uppsala, Sweden. Available at: https://www.sva.se/en/animals/wildlife/map-of-chronic-wasting-disease-cwd/ (accessed on April 14th, 2021).
Nei, M., Y. Suzuki and M. Nozawa (2010). "The Neutral Theory of Molecular Evolution in the Genomic Era." Annual Review of Genomics and Human Genetics **11**(1): 265-289.
Nico, P. B. C., F. de-Paris, E. R. Vinadé, O. B. Amaral, I. Rockenbach, B. L. Soares, R. Guarnieri, L. Wichert-Ana, F. Calvo, R. Walz, I. Izquierdo, A. C. Sakamoto, R. Brentani, V. R. Martins and M. M. Bianchin (2005). "Altered behavioural response to acute stress in mice lacking cellular prion protein." Behavioural Brain Research **162**(2): 173-181.
Niedziałkowska, M., K. J. Hundertmark, B. Jędrzejewska, K. Niedziałkowski, V. E. Sidorovich, M. Górny, R. Veeroja, E. J. Solberg, S. Laaksonen, H. Sand, V. A. Solovyev, M. Shkvyria, J. Tiainen, I. M. Okhlopkov, R. Juškaitis, G. Done, V. A. Borodulin, E. A. Tulandin and W. Jędrzejewski (2014). "Spatial structure in European moose (*Alces alces*): genetic data reveal a complex population history." Journal of Biogeography **41**(11): 2173-2184.
Nonno, R., M. A. Di Bari, L. Pirisinu, C. D'Agostino, I. Vanni, B. Chiappini, S. Marcon, G. Riccardi, L. Tran, T. Vikøren, J. Våge, K. Madslien, G. Mitchell, G. C. Telling, S. L. Benestad and U. Agrimi (2020). "Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America." Proceedings of the National Academy of Sciences **117**(49): 31417-31426.
Norstrom, E. M. and J. A. Mastrianni (2005). "The AGAAAAGA Palindrome in PrP Is Required to Generate a Productive PrP^{Sc}-PrP^{C} Complex That Leads to Prion Propagation." Journal of Biological Chemistry **280**(29): 27236-27243.
O'Rourke, K. I., T. R. Spraker, L. K. Hamburg, T. E. Besser, K. A. Brayton and D. P. Knowles (2004). "Polymorphisms in the prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer." Journal of General Virology **85**(5): 1339-1346.
O'Rourke, K. I., T. R. Spraker, D. Zhuang, J. J. Greenlee, T. E. Gidlewski and A. N. Hamir (2007). "Elk with a long incubation prion disease phenotype have a unique PrP^{d} profile." Neuroreport **18**(18): 1935-1938.

O'Rourke, K. I., T. E. Besser, M. W. Miller, T. F. Cline, T. R. Spraker, A. L. Jenny, M. A. Wild, G. L. Zebarth and E. S. Williams (1999). "PrP genotypes of captive and free-ranging Rocky Mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease." Journal of General Virology **80**(10): 2765-2679.
Oesch, B., D. Westaway, M. Wälchli, M. P. McKinley, S. B. H. Kent, R. Aebersold, R. A. Barry, P. Tempst, D. B. Teplow, L. E. Hood, S. B. Prusiner and C. Weissmann (1985). "A cellular gene encodes scrapie PrP 27-30 protein." Cell **40**(4): 735-746.
World Organisation for Animal Health (OIE) (2016). – Report of the anual meeting of the OIE Working Group on Wildlife. OIE Specialised commissions and groups. Paris, France. Available at: https://www.oie.int/en/standard-setting/specialists-commissions-working-ad-hoc-groups/working-groups-reports/working-group-on-wildlife/group-meetings-reports/ (accessed on April 14th, 2021).
World Organisation for Animal Health (OIE) (2017). Final Report of the 85th General Session. OIE. 85th General Session, Paris, France. Available at: http://www.oie.int/en/about-us/key-texts/final-reports-of-the-general-session/ (accessed on April 14th, 2021).
World Organisation for Animal Health (OIE) (2018). Final Report of the 86th General Session. OIE. 86th General Session, Paris, France. Available at: https://www.oie.int/en/about-us/key-texts/final-reports-of-the-general-session/ (accessed on April 14th, 2021).
Olszowy, K. M., J. Lavelle, K. Rachfal, S. Hempstead, K. Drouin, J. M. Darcy, C. Reiber and R. M. Garruto (2014). "Six-year follow-up of a point-source exposure to CWD contaminated venison in an Upstate New York community: risk behaviours and health outcomes 2005–2011." Public Health **128**(9): 860-868.
Otero, A., C. Duque Velásquez, C. Johnson, A. Herbst, R. Bolea, J. J. Badiola, J. Aiken and D. McKenzie (2019). "Prion protein polymorphisms associated with reduced CWD susceptibility limit peripheral PrP^{CWD} deposition in orally infected white-tailed deer." BMC Veterinary Research **15**(1): 50.
Palmer, M. S., A. J. Dryden, J. T. Hughes and J. Collinge (1991). "Homozygous prion protein genotype predisposes to sporadic Creutzfeldt–Jakob disease." Nature **352**(6333): 340-342.
Papassotiropoulos, A., M. A. Wollmer, A. Aguzzi, C. Hock, R. M. Nitsch and D. J.-F. de Quervain (2005). "The prion gene is associated with human long-term memory." Human Molecular Genetics **14**(15): 2241-2246.
Pape, W., J. Forster, C. Anderson, P. Bosque, P. Bosque and M. Miller (2006). "Human Prion Disease and Relative Risk Associated with Chronic Wasting Disease." Emerging Infectious Disease journal **12**(10): 1527-1535.
Pattison, I. H. (1965). Experiments with Scrapie with Special Reference to the Nature of the Agent and the Pathology of the Disease. N. I. o. N. D. a. Blindness. Washington, D.C., U. S. Government Printing Office. **2:** 249-257.

Peletto, S., M. Perucchini, C. Acín, M. P. Dalgleish, H. W. Reid, R. Rasero, P. Sacchi, P. Stewart, M. Caramelli, E. Ferroglio, E. Bozzetta, D. Meloni, R. Orusa, S. Robetto, S. Gennero, W. Goldmann and P. L. Acutis (2009). "Genetic variability of the prion protein gene (*PRNP*) in wild ruminants from Italy and Scotland." J Vet Sci **10**(2): 115-120.
Pérez, D. R., F. F. Damberger and K. Wüthrich (2010). "Horse Prion Protein NMR Structure and Comparisons with Related Variants of the Mouse Prion Protein." Journal of Molecular Biology **400**(2): 121-128.
Perucchini, M., K. Griffin, M. W. Miller and W. Goldmann (2008). "PrP genotypes of free-ranging wapiti (*Cervus elaphus nelsoni*) with chronic wasting disease." Journal of General Virology **89**(5): 1324-1328.
Petersen, B.-S., B. Fredrich, M. P. Hoeppner, D. Ellinghaus and A. Franke (2017). "Opportunities and challenges of whole-genome and -exome sequencing." BMC Genetics **18**(1): 14.
Pirisinu, L., L. Tran, B. Chiappini, I. Vanni, M. A. DiBari, G. Vaccari, T. Vikøren, K. I. Madslien, J. Våge, T. Spraker, G. Mitchell, A. Balachandran, T. Baron, C. Casalone, C. M. Rolandsen, K. H. Røed, U. Agrimi, R. Nonno and S. L. Benestad (2018). "Novel Type of Chronic Wasting Disease Detected in Moose (*Alces alces*), Norway." Emerging Infectious Disease journal **24**(12): 2210-2218.
Pitarch, J. L., H. C. Raksa, M. C. Arnal, M. Revilla, D. Martínez, D. Fernández de Luco, J. J. Badiola, W. Goldmann and C. Acín (2018). "Low sequence diversity of the prion protein gene (*PRNP*) in wild deer and goat species from Spain." Veterinary Research **49**(1): 33.
Pitra, C., J. Fickel, E. Meijaard and C. Groves (2004). "Evolution and phylogeny of old world deer." Molecular Phylogenetics and Evolution **33**(3): 880-895.
Plummer, I. H., S. D. Wright, C. J. Johnson, J. A. Pedersen and M. D. Samuel (2017). "Temporal patterns of chronic wasting disease prion excretion in three cervid species." Journal of General Virology **98**(7): 1932-1942.
Premzl, M., M. Delbridge, J. E. Gready, P. Wilson, M. Johnson, J. Davis, E. Kuczek and J. A. Marshall Graves (2005). "The prion protein gene: Identifying regulatory signals using marsupial sequence." Gene **349**: 121-134.
Premzl, M. and V. Gamulin (2007). "Comparative genomic analysis of prion genes." BMC Genomics **8**(1): 1.
Prusiner, S. B. (1982). "Novel proteinaceous infectious particles cause scrapie." Science **216**(4542): 136-144.
Prusiner, S. B. (1998). "Prions." Proceedings of the National Academy of Sciences **95**(23): 13363-13383.
Prusiner, S. B., D. Groth, A. Serban, R. Koehler, D. Foster, M. Torchia, D. Burton, S. L. Yang and S. J. DeArmond (1993). "Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies." Proceedings of the National Academy of Sciences **90**(22): 10608-10612.

Prusiner, S. B., D. F. Groth, D. C. Bolton, S. B. Kent and L. E. Hood (1984). "Purification and structural studies of a major scrapie prion protein." Cell **38**(1): 127-134.
Prusiner, S. B., M. Scott, D. Foster, K.-M. Pan, D. Groth, C. Mirenda, M. Torchia, S.-L. Yang, D. Serban, G. A. Carlson, P. C. Hoppe, D. Westaway and S. J. DeArmond (1990). "Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication." Cell **63**(4): 673-686.
Race, B., K. Meade-White, R. Race and B. Chesebro (2009). "Prion Infectivity in Fat of Deer with Chronic Wasting Disease." Journal of Virology **83**(18): 9608-9610.
Race, B., K. D. Meade-White, M. W. Miller, K. D. Barbian, R. Rubenstein, G. LaFauci, L. Cervenakova, C. Favara, D. Gardner, D. Long, M. Parnell, J. Striebel, S. A. Priola, A. Ward, E. S. Williams, R. Race and B. Chesebro (2009). "Susceptibilities of Nonhuman Primates to Chronic Wasting Disease." Emerging Infectious Disease journal **15**(9): 1366-1376.
Race, B., K. Williams, C. D. Orrú, A. G. Hughson, L. Lubke and B. Chesebro (2018). "Lack of Transmission of Chronic Wasting Disease to Cynomolgus Macaques." Journal of Virology **92**(14): e00550-00518.
Rees, E. E., E. H. Merrill, T. K. Bollinger, Y. T. Hwang, M. J. Pybus and D. W. Coltman (2012). "Targeting the detection of chronic wasting disease using the hunter harvest during early phases of an outbreak in Saskatchewan, Canada." Preventive Veterinary Medicine **104**(1): 149-159.
Reimers, E., K. H. Røed and J. E. Colman (2012). "Persistence of vigilance and flight response behaviour in wild reindeer with varying domestic ancestry." Journal of Evolutionary Biology **25**(8): 1543-1554.
Rhyan, J. C., M. W. Miller, T. R. Spraker, M. McCollum, P. Nol, L. L. Wolfe, T. R. Davis, L. Creekmore and K. I. O'Rourke (2011). "Failure of Fallow Deer (*Dama dama*) to Develop Chronic Wasting Disease When Exposed to a Contaminated Environment and Infected Mule Deer (*Odocoileus hemionus*)." J Wildl Dis **47**(3): 739-744.
Riek, R., S. Hornemann, G. Wider, R. Glockshuber and K. Wüthrich (1997). "NMR characterization of the full-length recombinant murine prion protein, mPrP(23–231)." FEBS Letters **413**(2): 282-288.
Rivera, N. A., A. L. Brandt, J. E. Novakofski and N. E. Mateus-Pinilla (2019). "Chronic Wasting Disease In Cervids: Prevalence, Impact And Management Strategies." Veterinary medicine (Auckland, N.Z.) **10**: 123-139.
Robinson, S. J., M. D. Samuel, C. J. Johnson, M. Adams and D. I. McKenzie (2012). "Emerging prion disease drives host selection in a wildlife population." Ecological Applications **22**(3): 1050-1059.
Robinson, S. J., M. D. Samuel, K. I. O'Rourke and C. J. Johnson (2012). "The role of genetics in chronic wasting disease of North American cervids." Prion **6**(2): 153-162.

Rongyan, Z., L. Xianglong, L. Lanhui, L. Xiangyun and F. Fujun (2008). "Evolution and Differentiation of the Prion Protein Gene (*PRNP*) among Species." Journal of Heredity **99**(6): 647-652.
Rujescu, D., A. M. Hartmann, C. Gonnermann, H. J. Möller and I. Giegling (2003). "M129V variation in the prion protein may influence cognitive performance." Molecular Psychiatry **8**(11): 937-941.
Røed, K. H. (1998). "Microsatellite Variation in Scandinavian Cervidae Using Primers Derived from Bovidae." Hereditas **129**(1): 19-25.
Røed, K. H., I. Bjørklund and B. J. Olsen (2018). "From wild to domestic reindeer – Genetic evidence of a non-native origin of reindeer pastoralism in northern Fennoscandia." Journal of Archaeological Science: Reports **19**: 279-286.
Røed, K. H., Ø. Flagstad, M. Nieminen, Ø. Holand, M. J. Dwyer, N. Røv and C. Vilà (2008). "Genetic analyses reveal independent domestication origins of Eurasian reindeer." Proceedings of the Royal Society B: Biological Sciences **275**(1645): 1849-1855.
Røed, K. H. and L. Midthjell (1998). "Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids." Molecular Ecology **7**(12): 1773-1776.
Sailer, A., H. Büeler, M. Fischer, A. Aguzzi and C. Weissmann (1994). "No propagation of prions in mice devoid of PrP." Cell **77**(7): 967-968.
Salanti, G., G. Amountza, E. E. Ntzani and J. P. A. Ioannidis (2005). "Hardy–Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power." European Journal of Human Genetics **13**: 840–848.
Salvesen, Ø., A. Espenes, M. R. Reiten, T. T. Vuong, G. Malachin, L. Tran, O. Andréoletti, I. Olsaker, S. L. Benestad, M. A. Tranulis and C. Ersdal (2020). "Goats naturally devoid of PrP^C are resistant to scrapie." Veterinary Research **51**(1): 1.
Saunders, S., S. Bartelt-Hunt and J. Bartz (2012). "Occurrence, Transmission, and Zoonotic Potential of Chronic Wasting Disease." Emerging Infectious Disease journal **18**(3): 369.
Schaid, D. J. (2004). "Evaluating associations of haplotypes with traits." Genetic Epidemiology **27**(4): 348-364.
Schauber, E. M. and A. Woolf (2003). "Chronic Wasting Disease in Deer and Elk: A Critique of Current Models and Their Application." Wildlife Society Bulletin **31**(3): 610-616.
Scheckel, C. and A. Aguzzi (2018). "Prions, prionoids and protein misfolding disorders." Nature Reviews Genetics **19**(7): 405-418.
Schmitz, M., S. Zafar, C. J. Silva and I. Zerr (2014). "Behavioral abnormalities in prion protein knockout mice and the potential relevance of PrP^C for the cytoskeleton." Prion **8**(6): 381-386.

Seabury, C. M., C. A. Gill, J. W. Templeton, J. B. Dyar, J. N. Derr, D. L. Adelson, E. Owens, D. S. Davis, D. C. Kraemer and J. E. Womack (2007). "Molecular Characterization of the Rocky Mountain Elk (*Cervus elaphus nelsoni*) *PRNP* Putative Promoter." Journal of Heredity **98**(7): 678-686.
Seabury, C. M., D. L. Oldeschulte, E. K. Bhattarai, D. Legare, P. J. Ferro, R. P. Metz, C. D. Johnson, M. A. Lockwood and T. A. Nichols (2020). "Accurate Genomic Predictions for Chronic Wasting Disease in U.S. White-Tailed Deer." G3: Genes, Genomes, Genetics: g3.401002.402019.
Sigurdson, C. J., E. S. Williams, M. W. Miller, T. R. Spraker, K. I. O'Rourke and E. A. Hoover (1999). "Oral transmission and early lymphoid tropism of chronic wasting disease PrP^{res} in mule deer fawns (*Odocoileus hemionus*)." Journal of General Virology **80**(10): 2757-2764.
Sigurdsson, B. (1954). "RIDA, A Chronic Encephalitis of Sheep: With General Remarks on Infections Which Develop Slowly and Some of Their Special Characteristics." British Veterinary Journal **110**(9): 341-354.
Simonic, T., S. Duga, B. Strumbo, R. Asselta, F. Ceciliani and S. Ronchi (2000). "cDNA cloning of turtle prion protein." FEBS Letters **469**(1): 33-38.
Skog, A., F. E. Zachos, E. K. Rueness, P. G. D. Feulner, A. Mysterud, R. Langvatn, R. Lorenzini, S. S. Hmwe, I. Lehoczky, G. B. Hartl, N. C. Stenseth and K. S. Jakobsen (2009). "Phylogeography of red deer (*Cervus elaphus*) in Europe." Journal of Biogeography **36**(1): 66-77.
Slapšak, U., G. Salzano, G. Ilc, G. Giachin, J. Bian, G. Telling, G. Legname and J. Plavec (2019). "Unique Structural Features of Mule Deer Prion Protein Provide Insights into Chronic Wasting Disease." ACS Omega **4**(22): 19913-19924.
Sohn, H.-J., J.-H. Kim, K.-S. Choi, J.-J. Nah, Y.-S. Joo, Y.-H. Jean, S.-W. Ahn, O.-K. Kim, D.-Y. Kim and A. Balachandran (2002). "A Case of Chronic Wasting Disease in an Elk Imported to Korea from Canada." Journal of Veterinary Medical Science **64**(9): 855-858.
Soldevila, M., A. M. Andrés, A. Ramírez-Soriano, T. Marquès-Bonet, F. Calafell, A. Navarro and J. Bertranpetit (2006). "The prion protein gene in humans revisited: Lessons from a worldwide resequencing study." Genome Research **16**(2): 231-239.
Speed, J. D. M., G. Austrheim, A. L. Kolstad and E. J. Solberg (2019). "Long-term changes in northern large-herbivore communities reveal differential rewilding rates in space and time." PLOS ONE **14**(5): e0217166.
Spooner, W., W. McLaren, T. Slidel, D. K. Finch, R. Butler, J. Campbell, L. Eghobamien, D. Rider, C. M. Kiefer, M. J. Robinson, C. Hardman, F. Cunningham, T. Vaughan, P. Flicek and C. C. Huntington (2018). "Haplosaurus computes protein haplotypes for use in precision drug design." Nature Communications **9**(1): 4128.

Spraker, T. R., A. Balachandran, D. Zhuang and K. I. O'Rourke (2004). "Variable patterns of distribution of PrP^{CWD} in the obex and cranial lympoid tissues of Rock Mountain elk (*Cervus elaphus nelsoni*) with subclinical chronic wasting disease." Veterinary Record **155**(10): 295-302.
Spraker, T. R., M. W. Miller, E. S. Williams, D. M. Getzy, W. J. Adrian, G. G. Schoonveld, R. A. Spowart, K. I. O'Rourke, J. M. Miller and P. A. Merz (1997). "Spongiform encephalopathy in free-ranging mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*) in northcentral Colorado." Journal of Wildlife Diseases **33**(1): 1-6.
Stockman, S. (1913). "Scrapie: An Obscure Disease of Sheep." Journal of Comparative Pathology and Therapeutics **26**: 317-327.
Strumbo, B., S. Ronchi, L. C. Bolis and T. Simonic (2001). "Molecular cloning of the cDNA coding for *Xenopus laevis* prion protein." FEBS Letters **508**(2): 170-174.
Swarbrick, P. A., A. B. Dietz, J. E. Womack and A. M. Crawford (1992). "Ovine and bovine dinucleotide repeat polymorphism at the MAF46 locus." Animal Genetics **23**(2): 182-182.
Tamgüney, G., M. W. Miller, L. L. Wolfe, T. M. Sirochman, D. V. Glidden, C. Palmer, A. Lemus, S. J. DeArmond and S. B. Prusiner (2009). "Asymptomatic deer excrete infectious prions in faeces." Nature **461**(7263): 529-532.
Tennant, J. M., M. Li, D. M. Henderson, M. L. Tyer, N. D. Denkers, N. J. Haley, C. K. Mathiason and E. A. Hoover (2020). "Shedding and stability of CWD prion seeding activity in cervid feces." PLOS ONE **15**(3): e0227094.
Tranulis, M. A., S. L. Benestad, T. Baron and H. Kretzschmar (2011). Atypical Prion Diseases in Humans and Animals. Prion Proteins. J. Tatzelt. Berlin, Heidelberg, Springer Berlin Heidelberg**:** 23-50.
Uerlings, S., V. Welter, B. Madea and M. Grabmüller (2021). "Comparative analysis of DNA extraction processes for DNA-based identification from putrefied bodies in forensic routine work." Forensic Science International **320**: 110707.
Valleron, A.-J., P.-Y. Boelle, R. Will and J.-Y. Cesbron (2001). "Estimation of Epidemic Size and Incubation Time Based on Age Characteristics of vCJD in the United Kingdom." Science **294**(5547): 1726-1728.
van Keulen, L. J., B. E. Schreuder, R. H. Meloen, G. Mooij-Harkes, M. E. Vromans and J. P. Langeveld (1996). "Immunohistochemical detection of prion protein in lymphoid tissues of sheep with natural scrapie." Journal of Clinical Microbiology **34**(5): 1228-1231.
van Rheede, T., M. M. W. Smolenaars, O. Madsen and W. W. de Jong (2003). "Molecular Evolution of the Mammalian Prion Protein." Molecular Biology and Evolution **20**(1): 111-121.

Vander Wal, E., D. Garant, S. Calmé, C. A. Chapman, M. Festa-Bianchet, V. Millien, S. Rioux-Paquette and F. Pelletier (2014). "Applying evolutionary concepts to wildlife disease ecology and management." Evol Appl(7): 856-868.
Vander Wal, E., P. C. Paquet and J. A. Andrés (2012). "Influence of landscape and social interactions on transmission of disease in a social cervid." Molecular Ecology **21**(5): 1271-1282.
Vikøren, T., J. Våge, K. I. Madslien, K. H. Røed, C. M. Rolandsen, L. Tran, P. Hopp, V. Veiberg, M. Heum, T. Moldal, C. G. d. Neves, K. Handeland, B. Ytrehus, Ø. Kolbjørnsen, H. Wisløff, R. Terland, B. Saure, K. M. Dessen, S. G. Svendsen, B. S. Nordvik and S. L. Benestad (2019). "First Detection of Chronic Wasting Disease in a Wild Red Deer (*Cervus elaphus*) in Europe." Journal of Wildlife Diseases **55**(4): 970-972.
Viljugrein, H., P. Hopp, S. L. Benestad, E. B. Nilsen, J. Våge, S. Tavornpanich, C. M. Rolandsen, O. Strand and A. Mysterud (2019). "A method that accounts for differential detectability in mixed samples of long-term infections with applications to the case of chronic wasting disease in cervids." Methods in Ecology and Evolution **10**(1): 134-145.
Vitenskapskomiteen for mattrygghet (VKM) (2016). – CWD in Norway. Opinion of the Panel on Biological Hazards. ISBN: 978-82-8259-216-1. Oslo, Norway. Available at: https://vkm.no/english/riskassessments/allpublications/unknownoriginofchronicwastingdiseaseinnorway.4.2375207615dac0245aee41ec.html (accessed on April 14th, 2021).
Vitenskapskomiteen for mattrygghet (VKM) (VKM) (2017). – CWD – update statement. Opinion of the Panel on Biological Hazards. ISBN: 978-82-8259-283-3. Oslo. Available at: https://vkm.no/english/riskassessments/allpublications/stilllowprobabilitythathumanscanbeinfectedwithchronicwastingdiseasecwd.4.4fdace2015eceedf2144d708.html (accessed on April 14th, 2021).
Vitenskapskomiteen for mattrygghet (VKM) (2017). – CWD in Norway – a state of emergency for the future of cervids (Phase II). Opinion of the Panel on Biological Hazards. ISBN: 978-82-8259-266-6. Oslo, Norway. Available at: https://vkm.no/english/riskassessments/allpublications/comprehensivemeasuresneededtoeradicatechronicwastingdiseaseinnorway.4.2375207615dac0245aee1882.html (accessed on April 14th, 2021).
Norwegian Veterinary Institute (Våge, J., P. Hopp, T. Vikøren, K. Madslien, A. Tarpai, T. Moldal and S. L. Benestad) (2020). – The surveillance programme for Chronic Wasting Disease (CWD) in free-ranging and captive cervids in Norway 2019. Surveillance programmes in Norway. ISSN: 1894-5678. Oslo, Norway. Available at: https://www.vetinst.no/en/surveillance-programmes/chronic-wasting-disease (accessed on April 14th, 2021).

Wadsworth, J. D. F., E. A. Asante, M. Desbruslais, J. M. Linehan, S. Joiner, I. Gowland, J. Welch, L. Stone, S. E. Lloyd, A. F. Hill, S. Brandner and J. Collinge (2004). "Human Prion Protein with Valine 129 Prevents Expression of Variant CJD Phenotype." Science **306**(5702): 1793-1796.
Wadsworth, J. D. F., S. Joiner, J. M. Linehan, K. Jack, H. Al-Doujaily, H. Costa, T. Ingold, M. Taema, F. Zhang, M. K. Sandberg, S. Brandner, L. Tran, T. Vikøren, J. Våge, K. Madslien, B. Ytrehus, S. L. Benestad, E. A. Asante and J. Collinge (2021). "Humanized Transgenic Mice Are Resistant to Chronic Wasting Disease Prions From Norwegian Reindeer and Moose." The Journal of Infectious Diseases: jiab033.
Walther, I., A. Staskevicius, A. Soutyrine and G. Mitchell (2019). Diagnosis of chronic wasting disease in a herd of farmed red deer (*Cervus elaphus*).
Wang, F., S. Yin, X. Wang, L. Zha, M.-S. Sy and J. Ma (2010). "Role of the Highly Conserved Middle Region of Prion Protein (PrP) in PrP−Lipid Interaction." Biochemistry **49**(37): 8169-8176.
White, S. N., T. R. Spraker, J. O. Reynolds and K. I. O'Rourke (2010). "Association analysis of *PRNP* gene region with chronic wasting disease in Rocky Mountain elk." BMC Research Notes **3**(1): 314.
Wiehler, J. and R. Tiedemann (1998). "Phylogeography of the European roe deer *Capreolus capreolus* as revealed by sequence analysis of the mitochondrial Control Region." Acta Theriologica: 187-197.
Wik, L., S. Mikko, M. Klingeborn, M. Stéen, M. Simonsson and T. Linné (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**(3): 256-260.
Wilesmith, J., J. Ryan and M. Atkinson (1991). "Bovine spongiform encephalopathy: epidemiological studies on the origin." Veterinary Record **128**(9): 199-203.
Wilkinson, D. M. (2017). Dispersal: Biogeography. eLS**:** 1-7.
Williams, E. S. and M. W. Miller (2002). "Chronic wasting disease in deer and elk in North America " Revue Scientifique et Technique **21**(2): 305-316.
Williams, E. S., D. O'Toole, M. W. Miller, T. J. Kreeger and J. E. Jewell (2018). "Cattle (*Bos taurus*) resist Chronic Wasting Disease following oral inoculation challenge or ten years' natural exposure in contaminated environments." Journal of Wildlife Diseases **54**(3): 460-470.
Williams, E. S. and S. Young (1980). "CHRONIC WASTING DISEASE OF CAPTIVE MULE DEER: A SPONGIFORM ENCEPHALOPATHY." Journal of Wildlife Diseases **16**(1): 89-98.
Williams, E. S. and S. Young (1982). "SPONGIFORM ENCEPHALOPATHY OF ROCKY MOUNTAIN ELK." Journal of Wildlife Diseases **18**(4): 465-471.

Wilson, G. A., S. M. Nakada, T. K. Bollinger, M. J. Pybus, E. H. Merrill and D. W. Coltman (2009). "Polymorphisms at the *PRNP* Gene Influence Susceptibility to Chronic Wasting Disease in Two Species of Deer (*Odocoileus* Spp.) in Western Canada." Journal of Toxicology and Environmental Health, Part A **72**(17-18): 1025-1029.
Wilson, G. A., C. Strobeck, L. Wu and J. W. Coffin (1997). "Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls." Molecular Ecology **6**(7): 697-699.
Wolfe, L. L., K. A. Fox and M. W. Miller (2014). ""Atypical" Chronic Wasting Disease in *PRNP* Genotype 225FF Mule Deer." Journal of Wildlife Diseases **50**(3): 660-665.
Wopfner, F., G. Weidenhöfer, R. Schneider, A. von Brunn, S. Gilch, T. F. Schwarz, T. Werner and H. M. Schätzl (1999). "Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein." Journal of Molecular Biology **289**(5): 1163-1178.
Wright, J. A., P. C. McHugh, M. Stockbridge, S. Lane, S. Kralovicova and D. R. Brown (2009). "Activation and repression of prion protein expression by key regions of intron 1." Cellular and Molecular Life Sciences **66**(23): 3809.
Yannic, G., L. Pellissier, J. Ortego, N. Lecomte, S. Couturier, C. Cuyler, C. Dussault, K. J. Hundertmark, R. J. Irvine, D. A. Jenkins, L. Kolpashikov, K. Mager, M. Musiani, K. L. Parker, K. H. Røed, T. Sipko, S. G. Þórisson, B. V. Weckworth, A. Guisan, L. Bernatchez and S. D. Côté (2014). "Genetic diversity in caribou linked to past and future climate change." Nature Climate Change **4**: 132–137.
Zachos, F. E., A. C. Frantz, R. Kuehn, S. Bertouille, M. Colyn, M. Niedziałkowska, J. Pérez-González, A. Skog, N. Spřěm and M.-C. Flamand (2016). "Genetic Structure and Effective Population Sizes in European Red Deer (*Cervus elaphus*) at a Continental Scale: Insights from Microsatellite DNA." Journal of Heredity **107**(4): 318-326.
Zheng, Z., M. Zhang, Y. Wang, R. Ma, C. Guo, L. Feng, J. Wu, H. Yao and D. Lin (2018). "Structural basis for the complete resistance of the human prion protein mutant G127V to prion disease." Scientific Reports **8**(1): 13211.
Zink, R. M., N. Najar, H. Vázquez-Miranda, B. L. Buchanan, D. Loy and B. W. Brodersen (2020). "Geographic variation in the *PRNP* gene and its promoter, and their relationship to chronic wasting disease in North American deer." Prion **14**(1): 185-192.
Ågren, E. O., K. Sörén, D. Gavier-Widén, S. L. Benestad, L. Tran, K. Wall, G. Averhed, N. Doose, J. Våge and M. Nöremark (2021). "First Detection of Chronic Wasting Disease in Moose (*Alces alces*) in Sweden." Journal of Wildlife Diseases **57**(2): 461-463.

10 Articles I - V

Article I

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ECOSPHERE

The demographic pattern of infection with chronic wasting disease in reindeer at an early epidemic stage

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Abstract. Infection patterns linked to age and sex are crucial to predict the population dynamic effects of diseases in long-lived species. How such demographic patterns of infection arise is often multifactorial, although the cause is commonly seen as a combination of immune status as well as variation in pathogen exposure. Prion diseases are particularly interesting, as they do not trigger an adaptive immune response; hence, differences in pathogen exposure linked to behavior could be the prime determinant of the pattern of infection. In cervids, the fatal prion disease, chronic wasting disease (CWD), is spreading geographically, with economic and cultural consequences in affected areas in North America, and all infected individuals eventually die from disease-associated sequelae if they live long enough. Understanding the causes of the demographic pattern of infection with CWD is therefore urgent but is limited by the fact that reported data primarily come from related deer species in North America. The recent (detected 2016) emergence of CWD among wild alpine reindeer (*Rangifer tarandus*) in Norway with a different social organization, that is, no home range behavior and no matrilineal female groups, offers an opportunity to advance our understanding of how behavior influences the infection patterns. Testing of 1081 males and 1278 females detected 19 animals positive for abnormal prion protein in brain and/or lymphatic tissues. No calves and only one male yearling were infected, with the remaining positives being adults (representing 1.5% of adult males and 0.5% of adult females). We found a strong sex-biased infection pattern in reindeer (with infection 2.7 times more likely in adult males), which is similar to the results reported in mule deer and white-tailed deer. The hazard of being detected as positive increased with age in males. There was no close genetic relatedness among positive animals. The results were consistent with the within-group contact of males being a possible major route of transmission. We discuss the demographic pattern of infection with CWD in view of the lack of stable home range behavior and other key behavioral traits of reindeer relevant to understanding pathogen exposure in general.

Key words: direct contact; disease ecology; environmental reservoirs; pathogen exposure; population dynamics; prions; social organization; transmission.

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Introduction

Parasites and pathogens are usually unevenly distributed among individuals in a population. Physiological and behavioral differences are expected to be major drivers of the skewed appearance of many diseases (Guerra-Silveira and Abad-Franch 2013). Differential investment in and development of parts of the immune system can create age- and sex-specific patterns of infection. A common pattern among mammals is higher infection levels in males compared to females (Schalk and Forbes 1997, Córdoba-Aguilar and Munguía-Steyer 2013, Metcalf and Graham 2018) and in the young and senescent compared to prime-aged individuals (Hayward et al. 2011, Abolins et al. 2018, Benton et al. 2018). However, there are many exceptions to these main demographic infection patterns (Vicente et al. 2007, Smyth and Drea 2016, Sparks et al. 2018). Differences in behavior and contact rates affect the likelihood of pathogen exposure, and such variability differs predictably between sexes and age classes (Smyth and Drea 2016, Silk et al. 2018). It is therefore often difficult to unravel the relative role of variation in immune defenses and pathogen exposure in the demographic patterns of disease infection.

Prion diseases are a particularly interesting group of diseases in this context, as prions do not trigger an adaptive immune response (Prusiner 1998). Chronic wasting disease (CWD) is a fatal neurodegenerative prion disease affecting cervids (Williams and Young 1992). Hence, the demographic pattern of CWD can shed light on the role of pathogen exposure as a basis upon which to understand demographic infection patterns of wildlife diseases in general. Chronic wasting disease was first observed in captive mule deer (*Odocoileus hemionus*) in the late 1960s in Colorado, USA. Chronic wasting disease has since spread to 26 states in the United States and reached three provinces of Canada. All individuals infected with CWD eventually die from the disease if they live long enough. The CWD epidemic has come to the point of causing population declines in white-tailed deer (*Odocoileus virginianus*; Edmunds et al. 2016) and mule deer (DeVivo et al. 2017) in some well-studied endemic areas. Understanding its demographic pattern of infection is crucial to understanding the population dynamic impact of CWD (Potapov et al. 2012, Samuel and Storm 2016). Further, the demographic pattern of infection can shed light on the mode of transmission (Potapov et al. 2013) and hence provide keys to mitigation.

There is a strong age-specific pattern of CWD infection (Samuel and Storm 2016). Calves are rarely found infected, and yearlings have less than half the chance of infection relative to that of adults (Miller and Conner 2005, Heisey et al. 2010, Samuel and Storm 2016). Chronic wasting disease has an incubation period of 1.5–2.5 yr in mule deer (Fox et al. 2006) and 2–5 yr in elk (*Cervus canadensis*), depending on the prion protein gene (PRNP) polymorphism (Moore et al. 2018). The lower infection prevalence in young animals probably results from the shorter time at risk of exposure combined with the lag between the time of prion infection and detection by standard diagnostic tests (Viljugrein et al. 2019). In both mule deer (Miller and Conner 2005) and white-tailed deer (Heisey et al. 2010), the prevalence of CWD was 2–3 times higher in males compared to females. Most likely, the sex effect is mainly driven by differences in pathogen exposure (Potapov et al. 2015) and, therefore, strongly depends on the social organization or behavior of a given species. However, our understanding of how the sex-specific infection pattern arises is limited by the fact that the data come from two closely related species, mule deer and white-tailed deer, while the most detailed demographic CWD infection studies of elk do not include males (Sargeant et al. 2011, Monello et al. 2014).

In 2016, the first natural cases of CWD in reindeer (*Rangifer tarandus*) and in Europe were reported (Benestad et al. 2016). The different social organizations of reindeer compared to other cervids offer a unique opportunity to learn more about the factors causing the demographic infection pattern of CWD and the general role of pathogen exposure. The lack of both matrilineal grouping and stable home range behavior in reindeer can shed light on the possible transmission routes and infection pattern. The population was surveyed for CWD during annual hunts in 2016 and 2017 and during population eradication that finished in April 2018 (Mysterud and Rolandsen

2018). We herein report the sex and age distribution of the reindeer positive for the abnormal prion protein (PrP^{Sc}) relative to the demographic composition of the population, and we estimate the genetic relatedness of positive individuals relative to the rest of the population. We test whether there is a sex bias in infection probability, as seen in mule deer and white-tailed deer, and whether infection probability increase with age among adults. If mother–offspring is the main route of transmission, we predict many infected individuals of 1.5–2.5 yr old, a time similar to the anticipated incubation period, and closer genetic similarity among the positives than expected from random in the population. Mule deer and white-tailed deer form matrilineal groups, resulting in higher infection levels among closely related females. In contrast, reindeer do not form similar matrilineal groups and relatedness is unlikely to increase horizontal contact rates required for prion transmission. We hence predict no stronger genetic relatedness among positive reindeer females than for a random sample of the population.

Materials and Methods

The study area

The data derive from the Nordfjella wild reindeer management area in the counties Sogn & Fjordane and Buskerud, Norway (between 60°37′–61°02′N and 07°14′–8°59′E). The Nordfjella area comprises a northern territory (zone 1) of approximately 2000 km^2 and a southern territory (zone 2) of approximately 1000 km^2, parted mainly due to a road (FV50 Hol-Aurland). Chronic wasting disease has only been detected in zone 1. The Nordfjella Mountains have a steep and rugged terrain. Most of the area is in the mid- and high-alpine zones above 1500 m a.s.l., with peaks extending to 1900 m a.s.l. This mountain range has a harsh and volatile climate due to the high elevation and it being situated on a climatic divide with a strong coastal influence in the west (wetter and warmer) and more of an inland climate in the east (drier and colder). The tree line is at approximately 800–1000 m a.s.l. The reindeer are alpine but occasionally use the surrounding birch (*Betula* spp.) forest, in particular during spring and early summer. During summer, over 60,000 domestic sheep (*Ovis aries*) graze in the area (VKM et al. 2018). Red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and moose (*Alces alces*) use the surrounding forests and, occasionally, the alpine habitat.

Reindeer data: sampling

In total, 2424 reindeer were tested for CWD in the Nordfjella reindeer management area, zone 1, in the period of March 2016–May 2018 (Appendix S1: Table S1). We excluded animals with unknown sex ($n = 94$) and/or unknown age class ($n = 68$) leaving 2365 reindeer (1085 males and 1280 females) for analysis (Table 1). The data originate from (1) hunting in 2016 (20 August–30 September), (2) extended hunting in 2017 (10 August–30 October), (3) culling (7 November 2017–1 May 2018) performed by marksmen, and (4) fallen stock from the index case in March 2016 to the last animal removal in May 2018 (Appendix S1: Table S1). Tissue samples for CWD testing were brain, as the *medulla oblongata*, and lymph nodes, which were mainly retropharyngeal (RLN), but in a few instances, mandibular lymph nodes or tonsil tissue was used. Hunters provided reindeer heads to be sampled by trained veterinarians, or in cases of culling, sampling was performed by the marksmen. Personnel sampling the tissues also provided jaws with teeth for age determination.

Testing for CWD

All brain and lymph node samples were sent to the Norwegian Veterinary Institute in Oslo for CWD testing. The primary test was an ELISA (TeSeE ELISA SAP; Bio-Rad, Hercules, California, USA) for the detection of PrP^{Sc}, hereafter designated prions. A positive or inconclusive result was confirmed by Western blot testing (TeSeE Western Blot, Bio-Rad). The analytical test sensitivity of the ELISA was evaluated by Hibler et al. (2003) to be 92.5% (81.8–97.9) for the obex (part of the brainstem) and 98.8% (93.5–99.97) for the RLN compared to immunohistochemistry of the same tissues. The analytical tests have close to perfect specificity (European Food Safety Authority [EFSA] 2005). Due to economic and logistical constraints, samples of RLN and brain tissue from the same individual were pooled in primary testing, slightly lowering the test sensitivity for RLN. More profound variation in the diagnostic sensitivity is due to individual variation in the stage of infection (Viljugrein et al. 2019).

Table 1. An overview of reindeer with known sex and age tested for CWD, by the presence of PrP^{Sc}, during the epidemic outbreak in the Nordfjella reindeer management area, zone 1, in Norway, 2016–18.

		Age																
Source	Sex	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Unknown	Sum
Hunt 2016	Males	40	13	16	19	13	15	3	5	3				1			1	21	150
	Females	36	20	35	20	15	6	9	8	7	6	4	1	2	1			3	173
Hunt 2017	Males	67	36	73	41	25	25	18	19	9	1	5		2				3	324
	Females	45	19	39	35	32	20	11	21	10	5	9	1	4	2	1	1	2	257
Marksmen 2017–18	Males	133	100	68	74	46	20	17	9	9	3	2						71	552
	Females	157	122	94	88	67	45	36	28	18	23	6	6	2	2	1	1	143	839
Fallen stock 2016–18	Males	1	1		1	3	1											48	55
	Females		3		1													5	9
Sum	Males	241	150	157	135	87	61	38	33	21	4	7		3			1	143	1081
	Females	238	164	168	144	114	71	56	57	35	34	19	8	8	5	2	2	153	1278
PrP^{Sc} positives	Males		1	2	3	2	2	1	1	1									13
	Females				3	3													6

Notes: In 2016, animals were not marked with zone and there may be included up to 35 hunted animals in 2016 from Nordfjella reindeer management area, zone 2. Note that exact age will differ depending on time of harvest. There were excluded 100 animals due to missing information on sex and/or age class, see Appendix S1: Table S1. For 87 of 479 calves (age 0) and 33 of 270 yearlings (age 1), the age class had been determined by the hunter and not been confirmed by official age determination.

Determination of the age

The standard procedure for aging reindeer in population surveillance programs at the Norwegian Institute for Nature Research is to separate calves and yearlings from older reindeer by tooth eruption patterns, while counting of cementum annuli in stained tooth sections is used to age older reindeer (Hamlin et al. 2000). In cases of uncertain counts of cementum annuli, a qualitative judgment of the mandible including the dentition pattern and wear is also used as a guide to ascertain the most likely correct age (Solberg et al. 2017). For the hunter harvest, half of the mandible was available. For the marksmen culling, only the incisive part was extracted and not the whole mandible. This may have made the separation of yearlings from older reindeer less accurate, but we do not expect any systematic over- or underestimation of age. We also note that the index case was aged based on tooth eruption and wear (as we did not receive the incisors from this reindeer) to be above 2.5 yr, probably 3–4 yr old (Benestad et al. 2016), and was thus concluded as 3 yr old in Table 1 and in the analysis. Age data were lacking from individuals found dead or injured (Appendix S1: Table S1).

Microsatellite marker analysis

Genomic DNA was isolated from brain samples from 19 cases and 41 controls using the DNeasy Blood and Tissue Kit (Qiagen, Oslo, Norway) as indicated by the manufacturer's protocol. Samples were analyzed for 18 microsatellite loci: NVHRT01, NVHRT03, NVHRT16, NVHRT31, NVHRT48, NVHRT66, NVHRT73, BM4513, BM6506, Oheq, DeerC89*, RT 1, RT 7, RT 9, RT 27, RT 30, OarFCB193, and MAF46 (Appendix S1: Table S2). The microsatellites were amplified in six-multiplex PCR using fluorescent-labeled forward primers (Appendix S1: Table S2). Each PCR contained 1.0 μL of genomic DNA as a template, 1 μL of dNTPs mix (4 × 2.5 mmol/L, VWR), 3 pmol of forward and reverse primers, 1.0 μL of Key Buffer (15 mmol/L $MgCl_2$, VWR), 0.05 μL of Taq DNA polymerase (5 U/μL, VWR), and purified water to a 10 μL volume. PCR conditions were set as an initial denaturation at 95°C for 2 min; then 26 amplification cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and finally, extension at 72°C for 10 min. The multiplex PCR products were pooled into three panels and run individually in a 3500xL Genetic Analyzer (Applied Biosystems, Schwerte, Germany). The fragment peaks were scored with GeneMapper version 5.0 (Applied Biosystems).

GenAlEx 6.5 (Peakall and Smouse 2012) and Arlequin v3.5 (Excoffier and Lischer 2010) were used to estimate genetic variation within and between cases and controls. The related package in R (Pew et al. 2015) was used to calculate the pairwise genetic relatedness between individuals

by Lynch and Ritland's (LR) method (Lynch and Ritland 1999). We tested whether individuals within cases and controls were more related than was randomly expected by permuting individuals between groups in 1000 iterations.

Statistical analysis

We used logistic regression to test for age- and sex-specific patterns of CWD infection in R vs. 3.5.1 (R Development Core Team 2018), using only data with known sex and age information (Table 1). Due to the low number of cases and slow epidemic development of CWD, we pooled data across years and included only adults (≥2 yr). We used the Akaike information criterion (AIC) to compare models.

We also ran a Cox regression (proportional-hazards regression) to estimate age- and sex-specific patterns of CWD infection. Cox regression is the most widely used method for modeling the relationship of covariates to a survival outcome (Therneau and Grambsch 2000). An advantage of the Cox model over ordinary regression models is that the inference procedures can easily handle right-censored responses, that is, cases in which individuals are removed from the study population before the event is observed. The coefficients in a Cox regression relate to hazard—a positive coefficient indicates a worse prognosis (shorter time to the event), and a negative coefficient indicates a protective effect of the variable with which it is associated, which, in our case, is the hazard of becoming infected. The hazard ratio associated with a predictor variable (multiplicative change in risk) is given by the exponent of its coefficient. We set the starting point of the observation period to the first month after the index case was reported (April 2016), as all animals in the population that were found dead or hunted were tested for PrP^{Sc} after the index case. The index case was therefore not included in the Cox regression. The study ended when the whole population was terminated and the last fallen stock from avalanches was tested (14 May 2018). Through the recruitment of calves, new animals were included in the study population during the time of study. In the Cox regression, we only included tested animals with known ages older than calves (Table 1). The potential covariates included were sex, age class (calves vs. yearlings or adults at the start of the study), and/or age (in years). Age class or age was included as the value the individual had at the time of inclusion in the study. In this analysis, we were assuming a calving date of 15 May for changing from one age/age class to another (Reimers 2002). To check for consistency, we repeated the analysis on the extended data set including 341 tested animals with known age classes but missing information on exact age. When age information was lacking for adults tested in 2017 or from the marksmen culling in 2017–2018, age classes at the time of inclusion in the study were imputed based on the category corresponding to the mean age of adults tested in 2017 or in the marksmen culling in 2017–2018.

Apparent (observed) prevalence is the proportion of animals from a representative sample of the population that are positive with the diagnostic method used (see Testing for CWD). Infected cases were modeled according to the hypergeometric distribution to obtain credibility intervals for the apparent prevalence. Population sizes were set to the total numbers hunted or found dead from the start of the hunting season in 2017 to the end of the marksmen culling. For this period, all adults, except 20–30 males and females, were registered tested at the Norwegian Veterinary Institute. Animals with unknown age class were distributed according to the population proportion of animals with known age classes. With perfect test specificity, true prevalence equals apparent prevalence divided by test sensitivity. We used a Bayesian framework to estimate the true (informed) prevalence from the apparent prevalence, taking into account the modeled diagnostic test sensitivity being dependent on stage of infection (Viljugrein et al. 2019). By simulating infected individuals of each age class with a random stage of infection, the modeled test sensitivity becomes a stochastic distribution and is dependent on assumed development of infection, the length of the incubation period (set as 2 yr), and tissue sampling regime (for details see Viljugrein et al. 2019). The stochastic distribution of the test sensitivity was accounted for by running our model in jags with r-package R2jags.

Results

Demographic infection pattern

A total of 19 animals with PrP^{Sc} out of 2359 tested reindeer were detected from 2016 to 2018

(Table 1; Appendix S1: Table S1). No calves and only one male yearling were found to be infected. The Bayesian apparent prevalence was 1.6% (95% credibility interval [CI] 1.4%, 1.8%) in adult males and 0.5% (95% CI 0.5%, 0.7%) in adult females in the last period from 10 August 2017 to 1 May 2018. The true prevalence that accounts for imperfect detectability with the given test regime was estimated as 1.8% (95% CI 1.5, 2.6) in adult males and 0.6% (95% CI 0.5%, 0.9%) in adult females. There was a strong male bias among infected reindeer, with 68.4% (13) being males and 31.6% (6) being females despite testing more females overall. Infection was detected among adult males of up to 8 yr of age (3.0% of males ≥5 yr old infected), whereas there was no positive among females 5 yr or older (Table 1). Among adults of known age (n = 1270), the logistic regression model confirmed that males were 2.7 (95% CI 1.0, 7.2) times more likely to test positive for PrP^{Sc} than were females (Z = 1.96, P = 0.05). The best-fit model only included sex and had a weight of evidence superior to the sex+age and age*sex models (Table 2).

The Cox proportional-hazards model confirmed effects of the sex and age categories on the hazard of being tested positive (Fig. 1A, Table 3, n = 1583). The hazard rate was approximately five times higher for males compared to females. The hazard rate of testing PrP^{Sc}-positive was higher for individuals who were already adults in the spring of 2016 compared to that of individuals maturing into the adult age class later in the study period. This may reflect that some adults were infected already at the onset of the observation period. The main results were robust upon extending the analysis to known age class (n = 1879). The model selection supported the inclusion of an age category term (Table 2). Models with ages in years or with an interaction term for sex and age in years resulted in less parsimonious models

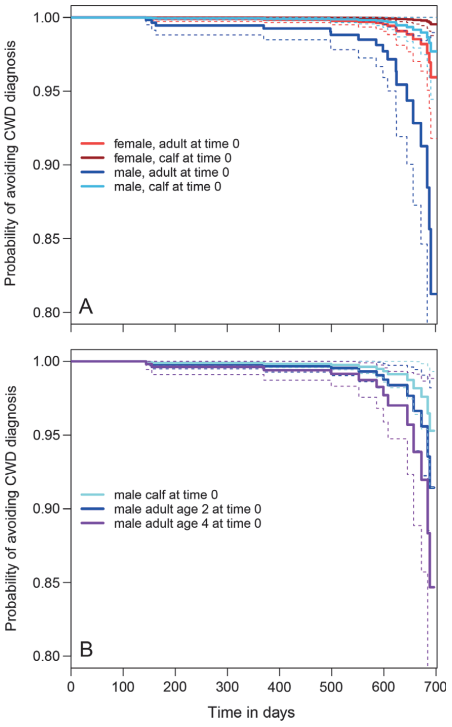

Fig. 1. The hazard of being tested PrP^{Sc}-positive of (A) male and female reindeer and (B) males of increasing ages from the Nordfjella population, zone 1, Norway, based on Cox regression models. The hazard was (A) higher in males than in females and (B) increased with age for males.

Table 2. Model selection with Akaike's information criterion (AIC) using logistic regression and Cox regression models to determine the age- and sex-specific pattern of CWD infection in reindeer from the Nordfjella reindeer area, zone 1, Norway, 2016–18.

Model parameters	AIC	ΔAIC
Logistic regression model		
Sex	188.85	0
Sex + age cat	190.78	1.93
Sex + age cat +sex:age cat	190.91	2.06
Cox proportional-hazards model		
Sex	225.30	7.01
Sex + age cat	218.29	0
Sex + age in years	221.89	3.60
Sex + age in years + sex:age	222.41	4.12
Age in years + (age in years)^2	217.69	−0.60
Males only		
Age in years	138.81	0
Age cat	141.10	2.29

Note: Age cat is age category (calf, yearling, and adult).

Table 3. Parameter estimates from the best Cox regression model of the hazard of being tested PrP^{Sc}-positive among reindeer from the Nordfjella reindeer area, zone 1, Norway, 2016–18.

Parameter	Coef.	SE (coef)	exp(coef)	Lower 0.95	Upper 0.95	*Z*	*P*
All							
Sex (male vs. female)	1.611	0.535	5.01	1.75	14.3	3.01	0.002
Yearlings vs. calves	1.571	0.871	4.81	0.87	26.5	1.80	0.071
Adults vs. calves	2.185	0.791	8.89	1.89	41.9	2.76	0.006
Males only							
Age in years	0.310	0.104	1.36	1.11	1.67	2.98	0.003

Note: The age parameter refers to the age class or age at the start of the study.

(Table 2). A model including age^2 was competitive ($\Delta AIC = -0.60$) but was only driven by the infected young females and did not fit the data for males. In a model on the male subset of the population, the hazard of testing PrP^{Sc}-positive over time increased with age (Fig. 1B, Table 3).

Genetic relatedness

The mean number of alleles was 6.4 (SE = 0.40) among the PrP^{Sc}-positive ($n = 19$) and 7.0 (SE = 0.51) among the PrP^{Sc}-negative ($n = 41$) reindeer. The mean observed heterozygosity was 0.722 (SE = 0.037) and 0.741 (SE = 0.025) for the two groups, respectively, while the mean expected heterozygosity was 0.741 (SE = 0.023) and 0.756 (SE = 0.018). There was no difference in genetic variation between the samples of positives and negatives ($F_{ST} = 0.000$, $P = 0.65$). The variation in relatedness estimators was similar for the cases ($n = 171$, mean = −0.022, SE = 0.007) and controls ($n = 820$, mean = −0.018, SE = 0.003). The mean relatedness within the groups was not higher than expected for the cases ($P < 0.959$) or for the controls ($P < 0.667$). The two positives with the closest genetic relatedness (0.388) differed in two microsatellite loci. These loci did not share alleles and were heterozygous in both animals, which suggests that these individuals could not have a parent–offspring relationship.

Discussion

The emergence of CWD in reindeer, which have a social organization contrasting from that of mule deer, white-tailed deer, and elk, offers an opportunity to learn more about how behavioral differences in pathogen exposure affect infection patterns. We found a 2.7-time higher infection rate in adult males compared to adult female reindeer, which is similar to the results reported in most mule deer and white-tailed deer populations (Miller and Conner 2005, Heisey et al. 2010, Rees et al. 2012, Samuel and Storm 2016). The current observations were consistent with frequent transmission in male–male groups at this expected early epidemic stage.

Demographic patterns of infection

Prion diseases, by the absence of an adaptive immune response, represent a rare case of how the demographic pattern of infection can arise from differences in pathogen exposure. For CWD, absent or low infection prevalence in calves and markedly lower infection prevalence in yearlings compared to adults have been documented for mule deer (Miller and Conner 2005), white-tailed deer (Heisey et al. 2010, Samuel and Storm 2016), and elk (Robinson et al. 2012, Monello et al. 2014, 2017). Our results in reindeer support this main pattern of prevalence across age classes. Prions are not detectable in early infection stages. The pattern of infection across age classes likely arises due to differences in the prion exposure period since birth and the long incubation period of the infection before it can be detected. Prevalence levels often continue to increase moderately with age in the adult stage, in particular, for males (Samuel and Storm 2016). A decline in infection among the oldest males was reported in both white-tailed deer and mule deer in Saskatchewan, Canada (Rees et al. 2012). We found an increasing hazard of becoming PrP^{Sc} infected with age in adult reindeer males (Fig. 1B).

All six PrP^{Sc}-positive females were 3–4 yr of age; however, females 5 yr and older comprised 41.0% of the adult (≥2 yr) females in the population. This clustering of infection in young adult females may be a random event, as the age and

sex interaction was not significant. Nevertheless, since the infected reindeer females were all 3–4 yr old, they were not old enough to be mothers of most PrP^{Sc} positives. Any such mother–offspring relations were denied by the confirmed lack of close genetic relatedness among the cases. We cannot exclude the possibility that older infected females died before sampling, but the low relatedness, together with the age distribution of all positives, suggests that mother–offspring contacts were not the main mode of transmission. Mother–offspring (vertical) transmission of CWD has been experimentally proven in muntjac (*Muntiacus reevesi*; Nalls et al. 2013), but horizontal transmission is regarded as the main mode of transmission among North American deer under natural conditions (Miller and Williams 2003). The PrP^{Sc}-positive prevalence of 1.5% in males and 0.5% in females indicates an early epidemic stage, and the demographic pattern of infection is consistent with mainly horizontal transmission of CWD.

Infection pattern and mode of transmission

Understanding transmission routes is critical for disease management, but establishing this information for CWD has proven difficult due to both direct transmission from animal to animal by contact with saliva, urine, or feces (Mathiason et al. 2006) and indirect transmission through environmental contamination (Miller et al. 2004). Direct contact is assumed as the main transmission route in the early epidemic stages of CWD and likely plays a near-constant role following behavior, throughout an epidemic, while environmental contamination becomes more important and increases transmission rates in later epidemic stages (Almberg et al. 2011). Female reindeer live in much larger groups than do males during the seasons in which they are sexually segregated. In the affected Nordfjella reindeer population in Norway, female groups were often in the range of 100–200, while male groups rarely exceeded 20–40 individuals. Hence, the broad levels of sociality and group sizes were poor predictors of the demographic infection pattern, suggesting that environmental contamination was not the primary mode of transmission, as expected in an early epidemic stage.

A largely unresolved issue in the CWD literature is the cause of the approximately 2–3 times higher infection prevalence in adult males than in females. This pattern was reported for white-tailed deer in Wisconsin (Heisey et al. 2010, Jennelle et al. 2014, Samuel and Storm 2016) and Illinois (Samuel and Storm 2016), for mule deer in Colorado (Miller and Conner 2005, Miller et al. 2008, Wolfe et al. 2018) and Wyoming (DeVivo et al. 2017), and for mule deer and white-tailed deer pooled in Saskatchewan, Canada (Rees et al. 2012). Female white-tailed and mule deer form matrilineal groups with stable home ranges, with minimum overlap with other matrilineal groups. There was a higher prevalence of CWD among genetically related females in the matrilineal social groups of both white-tailed deer (Grear et al. 2010) and mule deer (Cullingham et al. 2011) compared to unrelated females. The higher prevalence in adult males could be explained by males visiting many groups of females, increasing the overall likelihood of visiting an infected group (Grear et al. 2010). Reindeer are an interesting contrast, as they do not form matrilineal groups, nor do they use stable home ranges. Rather, their space use is characterized as being nomadic in large groups of related and nonrelated individuals. Hence, the strong sex bias in infection that also occurred in reindeer with this different spatial organization suggests that direct contact rates may be sufficiently frequent to yield a sex bias in CWD infection. The strongest associations in mule deer were among males pre-rut and between males and females during rut (Mejía-Salazar et al. 2017). Direct contact in the form required for pathogen transfer is most likely during male–male combat and female–male courtship (Potapov et al. 2013).

Interestingly, though the data published to date are limited, there appeared to be no sex bias in CWD infection in elk (Sargeant et al. 2011, Monello et al. 2014). The reasons for this are uncertain; however, one possible explanation is that environmental transmission may play a more important role in locations where sexual segregation is rather low and densities are high, such as winter ranges where elk may rut and spend the majority of the year (R. Monello, *personal communication*). Another main exception was white-tailed deer in Wyoming, with 28% of males and 42% of females being positive for CWD (Edmunds et al. 2016), suggestive that more environmental transmission in late

epidemic stages may erode the sex-specific infection in some areas.

Implications of the demographic infection pattern

Male-biased infections have several important implications. In polygynous species, males are not limiting for population growth unless sex ratios become extremely skewed (Mysterud et al. 2002). Any causes of mortality affecting adult females, however, are likely to have a strong impact on population growth (Gaillard et al. 1998). Therefore, the demographic patterns of parasites and disease may influence their impact on population dynamics (Miller et al. 2007). For CWD, the dynamic impact will be most strongly linked to the lowered survival of adult females. Infected females reproduce at close to normal rates until the late disease stages (Dulberger et al. 2010, Blanchong et al. 2012); hence, the effect of CWD on reproduction is expected to have a weaker impact on population dynamics. On the downside, males have a wider space use, and the male-biased infection may increase the chances of geographic spread. The harvesting of males can lead to more stable population dynamics under the threat of CWD (Jennelle et al. 2014), increase disease detection, and limit the risk of geographic spread (Lang and Blanchong 2012). This insight can guide the harvest management of adjacent populations with uncertain disease status in Norway and elsewhere.

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Literature Cited

Abolins, S., L. Lazarou, L. Weldon, L. Hughes, E. C. King, P. Drescher, M. J. O. Pocock, J. C. R. Hafalla, E. M. Riley, and M. Viney. 2018. The ecology of immune state in a wild mammal, *Mus musculus domesticus*. Plos Biology 16:e2003538.

Almberg, E. S., P. C. Cross, C. J. Johnson, D. M. Heisey, and B. J. Richards. 2011. Modeling routes of chronic wasting disease transmission: Environmental prion persistence promotes deer population decline and extinction. PLOS ONE 6:e19896.

Benestad, S. L., G. Mitchell, M. Simmons, B. Ytrehus, and T. Vikøren. 2016. First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer. Veterinary Research 47:88.

Benton, C. H., R. J. Delahay, F. A. P. Smith, A. Robertson, R. A. McDonald, A. J. Young, T. A. Burke, and D. Hodgson. 2018. Inbreeding intensifies sex- and age-dependent disease in a wild mammal. Journal of Animal Ecology 87:1500–1511.

Blanchong, J. A., D. A. Grear, B. V. Weckworth, D. P. Keane, K. T. Scribner, and M. D. Samuel. 2012. Effects of chronic wasting disease on reproduction and fawn harvest vulnerability in Wisconsin white-tailed deer. Journal of Wildlife Diseases 48:361–370.

Córdoba-Aguilar, A., and R. Munguía-Steyer. 2013. The sicker sex: understanding male biases in parasitic infection, resource allocation and fitness. PLOS ONE 8:e76246.

Cullingham, C. I., S. M. Nakada, E. H. Merrill, T. K. Bollinger, M. J. Pybus, and D. W. Coltman. 2011. Multiscale population genetic analysis of mule deer (*Odocoileus hemionus hemionus*) in western Canada sheds new light on the spread of chronic wasting disease. Canadian Journal of Zoology 89:134–147.

DeVivo, M. T., D. R. Edmunds, M. J. Kauffman, B. A. Schumaker, J. Binfet, T. J. Kreeger, B. J. Richards, H. M. Schätzl, and T. E. Cornish. 2017. Endemic chronic wasting disease causes mule deer population decline in Wyoming. PLOS ONE 12:e0186512.

Dulberger, J., N. T. Hobbs, H. M. Swanson, C. J. Bishop, and M. W. Miller. 2010. Estimating chronic wasting disease effects on mule deer recruitment and population growth. Journal of Wildlife Diseases 46:1086–1095.

Edmunds, D. R., M. J. Kauffman, B. A. Schumaker, F. G. Lindzey, W. E. Cook, T. J. Kreeger, R. G. Grogan, and T. E. Cornish. 2016. Chronic wasting disease drives population decline of white-tailed deer. PLOS ONE 11:e0161127.

European Food Safety Authority (EFSA). 2005. Scientific report of the European Food Safety Authority on the evaluation of rapid post mortem TSE tests intended for small ruminants. EFSA Journal 3:49.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567.

Fox, K. A., J. E. Jewell, E. S. Williams, and M. W. Miller. 2006. Patterns of PRP^{CWD} accumulation during the

course of Chronic Wasting Disease infection in orally inoculated mule deer (*Odocoileus hemionus*). Journal of General Virology 87:3451–3461.

Gaillard, J.-M., M. Festa-Bianchet, and N. G. Yoccoz. 1998. Population dynamics of large herbivores: variable recruitment with constant adult survival. Trends in Ecology and Evolution 13:58–63.

Grear, D. A., M. D. Samuel, K. T. Scribner, B. V. Weckworth, and J. A. Langenberg. 2010. Influence of genetic relatedness and spatial proximity on chronic wasting disease infection among female white-tailed deer. Journal of Applied Ecology 47:532–540.

Guerra-Silveira, F., and F. Abad-Franch. 2013. Sex bias in infectious disease epidemiology: patterns and processes. PLOS ONE 8:e62390.

Hamlin, K. L., D. F. Pac, C. A. Sime, R. M. DeSimone, and G. L. Dusek. 2000. Evaluating the accuracy of ages obtained by two methods for Montana ungulates. Journal of Wildlife Management 64:441–449.

Hayward, A. D., A. J. Wilson, J. G. Pilkington, T. H. Clutton-Brock, J. M. Pemberton, and L. E. B. Kruuk. 2011. Natural selection on a measure of parasite resistance varies across ages and environmental conditions in a wild mammal. Journal of Evolutionary Biology 24:1664–1676.

Heisey, D. M., E. E. Osnas, P. C. Cross, D. O. Joly, J. A. Langenberg, and M. W. Miller. 2010. Linking process to pattern: estimating spatiotemporal dynamics of a wildlife epidemic from cross-sectional data. Ecological Monographs 80:221–240.

Hibler, C. P., et al. 2003. Field validation and assessment of an enzyme-linked immunosorbent assay for detecting chronic wasting disease in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*). Journal of Veterinary Diagnostic Investigation 15:311–319.

Jennelle, C. S., V. Henaux, G. Wasserberg, B. Thiagarajan, R. E. Rolley, and M. D. Samuel. 2014. Transmission of chronic wasting disease in Wisconsin white-tailed deer: implications for disease spread and management. PLOS ONE 9:e91043.

Lang, K. R., and J. A. Blanchong. 2012. Population genetic structure of white-tailed deer: understanding risk of chronic wasting disease spread. Journal of Wildlife Management 76:832–840.

Lynch, M., and K. Ritland. 1999. Estimation of pairwise relatedness with molecular markers. Genetics 152:1753–1766.

Mathiason, C. K., et al. 2006. Infectious prions in the saliva and blood of deer with chronic wasting disease. Science 314:133.

Mejía-Salazar, M. F., A. W. Goldizen, C. S. Menz, R. G. Dwyer, S. P. Blomberg, C. L. Waldner, C. I. Cullingham, and T. K. Bollinger. 2017. Mule deer spatial association patterns and potential implications for transmission of an epizootic disease. PLOS ONE 12:e0175385.

Metcalf, C. J., and A. L. Graham. 2018. Schedule and magnitude of reproductive investment under immune trade-offs explains sex differences in immunity. Nature Communications 9:4391.

Miller, M. W., and M. M. Conner. 2005. Epidemiology of chronic wasting disease in free-ranging mule deer: spatial, temporal, and demographic influences on observed prevalence patterns. Journal of Wildlife Diseases 41:275–290.

Miller, M. W., H. M. Swanson, L. L. Wolfe, F. G. Quartarone, S. L. Huwer, C. H. Southwick, and P. M. Lukacs. 2008. Lions and prions and deer demise. PLOS ONE 3:e4019.

Miller, M. R., A. White, K. Wilson, and M. Boots. 2007. The population dynamical implications of male-biased parasitism in different mating systems. PLOS ONE 2:e624.

Miller, M. W., and E. S. Williams. 2003. Horizontal prion transmission in mule deer. Nature 425:35–36.

Miller, M. W., E. S. Williams, N. T. Hobbs, and L. L. Wolfe. 2004. Environmental sources of prion transmission in mule deer. Emerging Infectious Diseases 10:1003–1006.

Monello, R. J., N. L. Galloway, J. G. Powers, S. A. Madsen-Bouterse, W. H. Edwards, M. E. Wood, K. I. O'Rourke, and M. A. Wild. 2017. Pathogen-mediated selection in free-ranging elk populations infected by chronic wasting disease. Proceedings of the National Academy of Sciences of the United States of America 114:12208–12212.

Monello, R. J., J. G. Powers, N. T. Hobbs, T. R. Spraker, M. K. Watry, and M. A. Wild. 2014. Survival and population growth of a free-ranging elk population with a long history of exposure to chronic wasting disease. Journal of Wildlife Management 78:214–223.

Moore, S. J., C. E. Vrentas, S. Hwang, M. H. West Greenlee, E. M. Nicholson, and J. J. Greenlee. 2018. Pathologic and biochemical characterization of PrP^{Sc} from elk with *PRNP* polymorphisms at codon 132 after experimental infection with the chronic wasting disease agent. BMC Veterinary Research 14:80.

Mysterud, A., T. Coulson, and N. C. Stenseth. 2002. The role of males in the population dynamics of ungulates. Journal of Animal Ecology 71:907–915.

Mysterud, A., and C. M. Rolandsen. 2018. A reindeer cull to prevent chronic wasting disease in Europe. Nature Ecology and Evolution 2:1343–1345.

Nalls, A. V., et al. 2013. Mother to offspring transmission of chronic wasting disease in Reeves' muntjac deer. PLOS ONE 8:e71844.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics (Oxford, England) 28:2537–2539.

Pew, J., P. H. Muir, J. Wang, and T. R. Frasier. 2015. related: an R package for analysing pairwise relatedness from codominant molecular markers. Molecular Ecology Resources 15:557–561.

Potapov, A., E. Merrill, and M. A. Lewis. 2012. Wildlife disease elimination and density dependence. Proceedings of the Royal Society of London, Series B 279:3139–3145.

Potapov, A., E. Merrill, M. Pybus, D. Coltman, and M. A. Lewis. 2013. Chronic wasting disease: possible transmission mechanisms in deer. Ecological Modelling 250:244–257.

Potapov, A., E. Merrill, M. Pybus, and M. A. Lewis. 2015. Empirical estimation of R_0 for unknown transmission functions: the case of chronic wasting disease in Alberta. PLOS ONE 10:e0140024.

Prusiner, S. B. 1998. Prions. Proceedings of the National Academy of Sciences of the United States of America 95:13363–13383.

R Development Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rees, E. E., E. H. Merrill, T. K. Bollinger, Y. T. Hwang, M. J. Pybus, and D. W. Coltman. 2012. Targeting the detection of chronic wasting disease using the hunter harvest during early phases of an outbreak in Saskatchewan, Canada. Preventive Veterinary Medicine 104:149–159.

Reimers, E. 2002. Calving time and foetus growth among wild reindeer in Norway. Rangifer 22:61–66.

Robinson, S. J., M. D. Samuel, C. J. Johnson, M. Adams, and D. I. McKenzie. 2012. Emerging prion disease drives host selection in a wildlife population. Ecological Applications 22:1050–1059.

Samuel, M. D., and D. J. Storm. 2016. Chronic wasting disease in white-tailed deer: infection, mortality, and implications for heterogeneous transmission. Ecology 97:3195–3205.

Sargeant, G. A., D. C. Weber, and D. E. Roddy. 2011. Implications of chronic wasting disease, cougar predation, and reduced recruitment for elk management. Journal of Wildlife Management 75:171–177.

Schalk, G., and M. R. Forbes. 1997. Male biases in parasitism of mammals: effects of study type, host age, and parasite taxon. Oikos 78:67–74.

Silk, M. J., N. L. Weber, L. C. Steward, D. J. Hodgson, M. Boots, D. P. Croft, R. J. Delahay, and R. A. McDonald. 2018. Contact networks structured by sex underpin sex-specific epidemiology of infection. Ecology Letters 21:309–318.

Smyth, K. N., and C. M. Drea. 2016. Patterns of parasitism in the cooperatively breeding meerkat: a cost of dominance for females. Behavioral Ecology 27:148–157.

Solberg, E. J., et al. 2017. Cervids 1991-2016: summary report from the National Monitoring Program for wild cervids. (In Norwegian with English summary). NINA Rapport 1388:1–125.

Sparks, A. M., K. Watt, R. Sinclair, J. G. Pilkington, J. M. Pemberton, S. E. Johnston, T. N. McNeilly, and D. H. Nussey. 2018. Natural selection on anti-helminth antibodies in a wild mammal population. American Naturalist 192:745–760.

Therneau, T. M., and P. M. Grambsch. 2000. Modeling survival data: extending the Cox model. Springer, New York, New York, USA.

Vicente, J., L. Pérez-Rodríguez, and C. Gortazar. 2007. Sex, age, spleen size, and kidney fat of red deer relative to infection intensities of the lungworm *Elaphostrongylus cervi*. Naturwissenschaften 94:581.

Viljugrein, H., P. Hopp, S. L. Benestad, E. B. Nilsen, J. Våge, S. Tavornpanich, C. M. Rolandsen, O. Strand, and A. Mysterud. 2019. A method that accounts for differential detectability in mixed samples of long-term infections with applications to the case of chronic wasting disease in cervids. Methods in Ecology and Evolution 10:134–145.

VKM, et al. 2018. Factors that can contribute to spread of CWD - an update on the situation in Nordfjella, Norway. Opinion of the Panel on biological hazards. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

Williams, E. S., and S. Young. 1992. Spongiform encephalopathies in Cervidae. Revue Scientifique et Technique 11:551–567.

Wolfe, L. L., M. K. Watry, M. A. Sirochman, T. M. Sirochman, and M. W. Miller. 2018. Evaluation of a test and cull strategy for reducing prevalence of Chronice Wasting Disease in mule deer (*Odocoileus hemionus*). Journal of Wildlife Diseases 54:511–519.

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Article II

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Chronic wasting disease associated with prion protein gene (*PRNP*) variation in Norwegian wild reindeer (*Rangifer tarandus*)

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RESEARCH PAPER

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Chronic wasting disease associated with prion protein gene (*PRNP*) variation in Norwegian wild reindeer (*Rangifer tarandus*)

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ABSTRACT

The emergence of CWD in Europe in 2016 and the first natural infection in wild reindeer warranted disease management. This led to the testing of 2424 hunted or culled reindeer during 2016–2018, from the infected subpopulation in the Nordfjella mountain range in Southern Norway. To identify any association between *PRNP* variation and CWD susceptibility, we characterized the open reading frame of the *PRNP* gene in 19 CWD positive reindeer and in 101 age category- and sex-matched CWD negative controls. Seven variant positions were identified: 6 single nucleotide variants (SNVs) and a 24 base pair (bp) deletion located between nucleotide position 238 and 272, encoding four instead of five octapeptide repeats. With a single exception, all variant positions but one were predicted to be non-synonymous. The synonymous SNV and the deletion are novel in reindeer. Various combinations of the non-synonymous variant positions resulted in the identification of five *PRNP* alleles (A-E) that structured into 14 genotypes. We identified an increased CWD risk in reindeer carrying two copies of the most common allele, A, coding for serine in position 225 (Ser225) and in those carrying allele A together with the 24 bp deletion.

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Introduction

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) affecting ruminants of the *Cervidae* family [1], like Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, and scrapie in small ruminants. CWD expanded its geographic distribution and possibly its prion strain diversity with the emergence in Eurasian reindeer (*Rangifer tarandus*) [2] and moose [3] in Norway in 2016. Previously, this disease had been confined to North America and the Republic of Korea, reported for the first time in Colorado, USA in 1967. Among affected wild cervids in North America are mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus nelsoni*) and moose (*Alces alces*) [1]. Intriguingly, CWD has been identified in North American *Rangifer* only recently and in a captive reindeer [4], despite the potential overlap in cervid habitats. There is a single report of CWD detection in wild-red deer (*Cervus elaphus*) in Europe [5], even though former reports in captive herds [6,7].

CWD is caused by the conversion of the host-encoded cellular prion protein (PrP^{C}) into an abnormal isoform (PrP^{Sc}, also called prions) [8–10] of which accumulation eventually causes fatal neurodegeneration. Natural transmission and horizontal spread of prions occur in classical scrapie in sheep and CWD. Prions shed to the environment remain infectious for considerable periods [11–13].

Variation in *PRNP* (the gene encoding PrP^{C}), particularly within the open reading frame (ORF), is associated with the occurrence of prion disease and may affect prion strain characteristics [14]. Disease susceptibility and progression linked to *PRNP* variation has been reported in elk [15,16], mule deer [17] and white-tailed deer [18,19]. As reported [20–22] 20 known amino acid variant positions within the ORF of *PRNP* are known in cervids including elk, red deer, sika deer (*Cervus nippon*), fallow deer (*Dama dama*), white-tailed deer, mule deer, moose, Chinese water deer (*Hydropotes inermis*) and caribou (*Rangifer tarandus*).

Experiments have shown that reindeer can contract CWD after either inoculation with PrP^{Sc} from white-tailed deer or elk, or by co-housing with infected

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Supplemental data for this article can be accessed here.

reindeer. In addition, these experiments demonstrated that *PRNP* genotype probably affects disease susceptibility and progression [23,24].

The cases of CWD discussed here represent the first known naturally PrP^{Sc} infected reindeer; and all were detected in the Nordfjella mountain area, which is 1 of 23 wild reindeer management areas in Norway (Figure 1). Human infrastructures and reindeer migratory patterns divide Nordfjella into two zones (1 and 2), and the outbreak was limited to zone 1. As a result of the health, economic and biodiversity concerns related to possible spread of CWD from this area [25], the Norwegian government initiated measures to eradicate or at least halt further dispersion of the disease [26], i.e. eradication of the entire subpopulation of reindeer in Nordfjella zone 1 between 2016 and 2018 [27].

We here characterize the coding region of *PRNP* in 120 reindeer, including all 19 CWD cases and 101 controls matched for sex and age categories. The material was analysed for any association between genetic variation and the occurrence of PrP^{Sc}. This is the first report of *PRNP* genetic modulation of CWD risk within a reindeer population experiencing an outbreak of the disease. Data presented herein, will be relevant for disease management and allow crude estimation of disease susceptibility at a population-level.

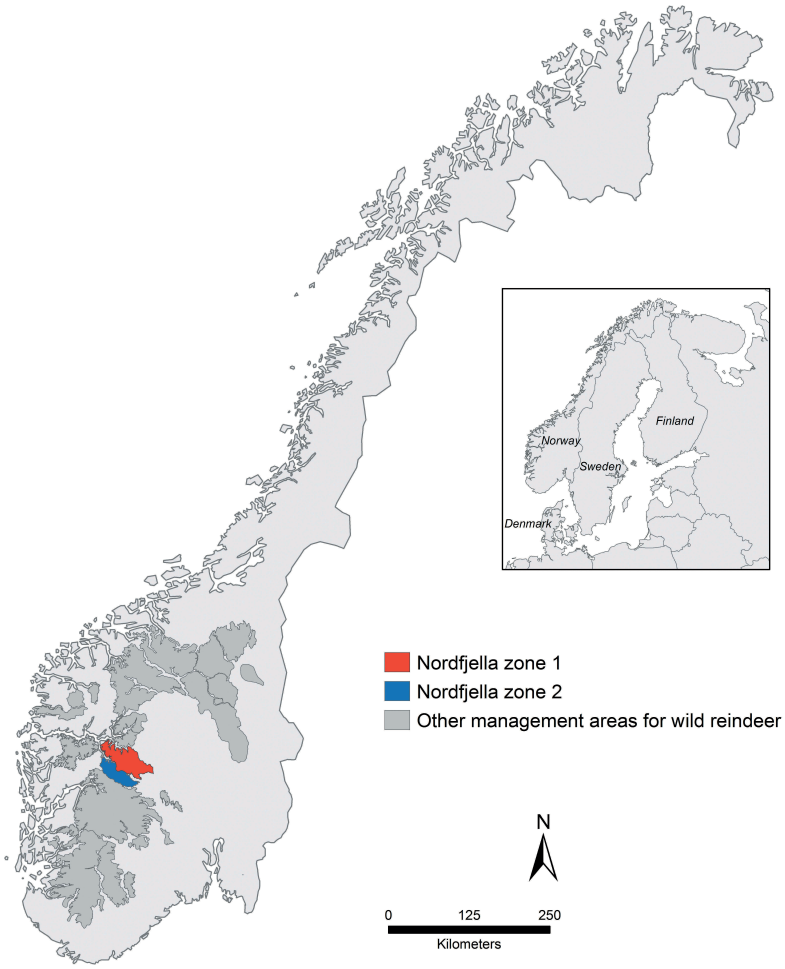

Figure 1. Localization of Nordfjella zones 1, 2 and other wild reindeer management areas in Southern Norway. All cases were detected in zone 1 and sampled in 2016–2018.

Results

PRNP variation in the study population

Sequencing of the ORF of *PRNP* (771 bp) revealed seven variant positions: six single nucleotide variants (SNVs) at positions 4, 6, 385, 505, 526 and 674; and a 24 bp deletion. With the exception of a synonymous substitution at position 6, all variant positions encoded amino acid changes. All variant positions were in Hardy–Weinberg Equilibrium (HWE) (*P*-value = 0.603–1.000). The deletion (249_272del) was located between nucleotide positions 238 and 272 and involved an octapeptide repeat element. Carriers of this deletion tested negative (no PCR product) for the presence of the *PRNP* pseudogene (*PRNPψ*), confirming the presence of the deletion within the functional *PRNP*. The SNV at position 6 and the 24 bp deletion have not been previously reported, other alleles were identical to published *Rangifer tarandus* sequences [21,28]. The sequence data were submitted to GenBank under the following accession numbers: MN784959 (*Rangifer tarandus tarandus* with 6G>A); MN784960 (*Rangifer tarandus tarandus* with 6G>A; 674C>A); MN784961 (*Rangifer tarandus tarandus* with 4G>A; 6G>A; 385G>A; 505G>A); MN784958 (*Rangifer tarandus tarandus* with 249_272del).

The non-synonymous variant sites served as markers to infer *PRNP* alleles encoding unique PrP in the study population. Pairwise analysis of linkage disequilibrium (LD) between 4G>A, 385G>A and 505G>A (D' = 0.999; r^2 = 0.999; *P*-value = <0.0001, Supplementary Figure 1), indicated that these positions are genetically linked. LD analyses for SNV at 674 with all other markers gave significantly high D' and low r^2 values suggesting that all are linked to 674C. The *PRNP* alleles (Table 1) were named according to amino acid substitution and codon number relative to reference sequence AAZ81474.1, i.e. allele A (Ser225), B (Tyr225), C (deletion), D (Asp176) and E (Met2.Ser129. Met169). Alleles A (Ser225) and B (Tyr225) represented the most common alleles within the study population (Table 1).

The five *PRNP* alleles (A-E) combined into 14 different genotypes of which A/B (27.5%) and A/A (20.8%) were the most common. Animals homozygous for allele C (deletion) were not observed. Other *PRNP* genotypes were observed at frequencies equal to or below 10%. Detailed descriptions of the variant positions and alleles are given in Supplementary Table 1 and Supplementary Table 2.

Cloning and sequencing of the *PRNP* coding sequence of three heterozygous animals with genotypes A/C and A/E confirmed the identity of the allele sequences separately.

Expression and Western blot of reindeer PrP

Human neuroblastoma (SH-SY5Y) cells expressing reindeer *PRNP* allele C (deletion; $rePrP^{del}$) and allele A (Ser225; $rePrP^{wt}$) produced similar amounts of PrP^C with a similar glycosylation pattern (Figure 2), indicating that PrP^C trafficking and post-translational modification with the attachment of N-glycans appears unaffected by the 24 bp deletion.

PRNP variation in cases and control-groups

The genotype frequencies within the controls were in HWE (*P*-value = 1.000). Only four genotypes were identified among cases, whereas all 14 were represented in the controls (Figure 3). Alleles A (Ser225), B (Tyr225) and C (deletion) were identified in both groups, whereas alleles D (Asp176) and E (Met2.Ser129. Met169) were only detected in controls (Figure 4). *PRNP* allele and genotype frequencies were statistically different between cases and controls (Fisher's exact test: *P* = 3.59e-05 and 0.01, respectively).

Allele A (Ser225) was the most prevalent in both groups, with a frequency of 73.7% in cases and 41.1% in controls. Similarly, allele C (deletion) was also more prevalent (13.2%) in infected than in non-infected (4.5%). In

Table 1. *PRNP* coding sequence alleles and frequencies in Norwegian wild reindeer from Nordfjella zone 1. The allele represents the DNA arrangement within the *PRNP* coding sequence, constructed by phasing non-synonymous variant positions identified in the study population. Variant positions are given at the nucleotide and protein level. Listed positions are characteristic nucleotides and codons for each *PRNP* allele, otherwise identical to reference sequence DQ154293.1 (nucleotide) and AAZ81474.1 (protein). Abbreviations: *PRNP* = prion protein gene; n = number of alleles in the study population.

	PRNP open reading frame variant positions						
Nucleotide Protein	4G>A Val2Met	249_272del Trp84_Gly91del	385G>A Gly129Ser	505G>A Val169Met	526A>G Asn176Asp	674C>A Ser225Tyr	Study population *n* = 240
Allele nomenclature							
A	Val	Trp84_Gly91	Gly	Val	Asn	Ser	111 (46.3%)
B	-	-	-	-	-	Tyr	73 (30.4%)
C	-	Trp84_Gly91del	-	-	-	-	23 (9.6%)
D	-	-	-	-	Asp	-	19 (7.9%)
E	Met	-	Ser	Met	-	-	14 (5.8%)

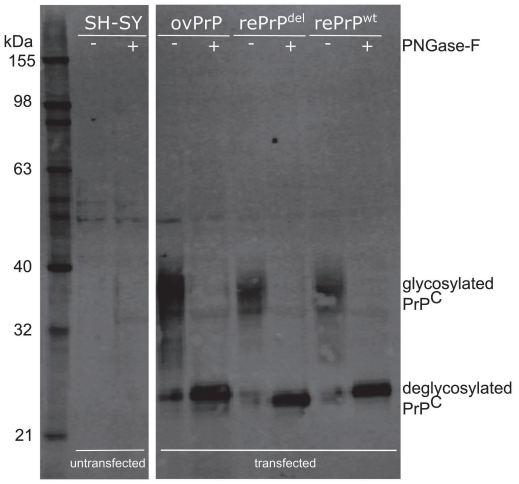

Figure 2. Western blot analysis of PrP^{C} transiently expressed in human neuroblastoma SH-SY5Y cells. Samples were untreated (-) or deglycosylated by PNGase-F treated (+) SH-SY5Y cells, untransfected (SH-SY) and transfected clones with ovine *PRNP* (ovPrP), reindeer *PRNP* with 24 bp deletion (rePrP^{del}) and wild type reindeer *PRNP* (rePrP^{wt}). Deglycosylated bands from rePrP^{del} and rePrP^{wt} differ with 1 kDa as expected. The membrane was probed with anti-PrP mab P4.

contrast, allele B (Tyr225) was more frequent in controls (33.7%) compared to cases (13.2%) (Figure 4). Of the common genotypes, A/A at 52.6% was the most prevalent among cases, contrasting with 14.9% in controls amongst which the A/B genotype was the most frequent (28.7%) (Figure 3).

Of the *PRNP* genotypes found exclusively in controls (Figure 3), B/B was the most frequent and served as the baseline genotype for the regression analysis. Firth logistic regression analysis suggested an increase in CWD risk on the shift from B/B to either A/A or A/C (Supplementary Table 3). Given the significance of genotypes A/A and A/C as predictors of CWD, we performed a similar regression analysis to test the effect of copy number of these alleles and CWD status. The model revealed that carrying two copies of allele A (Ser225) and/or allele C (deletion) is a risk factor for CWD, significantly increasing the odds ratio (O.R.) (O.R: 42.39; 95% C.I. = 5.12–5534.03) (Table 2).

Discussion

This is the first study to analyse *PRNP* coding variation among cases and controls in a population of wild reindeer sampled during an outbreak of CWD. Our data show that of the five alleles detected (A-E), A (Ser225) and C (deletion) were overrepresented among the cases and that all affected reindeer carried allele A, allele C or both. Allele C differs only from allele A in the octapeptide deletion. By comparing the expression of alleles A (rePrP^{wt}) and C (rePrP^{del}) in transfected cells, we showed as expected, that loss of a single octarepeat does not affect translation of *PRNP* mRNA or further post-translational protein modification with the attachment of N-glycans. Both alleles A (Ser225) and C (deletion) were overrepresented among the cases when compared to the controls, almost twice and treble, respectively. Given their overrepresentation in the CWD-affected reindeer, we combined the two alleles when performing the Firth logistic regression analysis, which returned a high odds ratio, indicating an increased CWD risk.

The allele B (Tyr225) carrying the Ser225Tyr substitution was more frequent among controls compared to cases. An analogous substitution (Ser225Phe) in wild mule deer results in a longer CWD incubation time in heterozygotes [29]. This clinical onset likely relates to the structural effect Ser225Phe has on PrP, that is proposed delay of PrP^{C}-to-PrP^{Sc} conversion [30], a feature observed *in vitro* for Ser225Tyr [31].

Alleles D (Asp176) and E (Met2.Ser129.Met169) were not detected among cases but constituted 20.8% of all alleles identified in controls. In sheep, a similar substitution at codon 176 (Asn176Lys) is considered protective against classical scrapie challenge [32,33]. Likewise, reindeer carrying a single copy of the allele E (Met2.Ser129.Met169) did not develop CWD [23] after oral challenge. In Moore et al., reindeer carrying allele E had longer survival-times following intracranial exposure [24]. In the same experiment, a reindeer with a genotype carrier of E, found dead without showing clinical signs ~13 months post-intracranial inoculation, had no histopathological lesions or PrP^{Sc} deposition at post-mortem examination.

The different distribution of the alleles by CWD status is in accordance with *in vivo* and *in vitro* data comparing the allele A (Ser225) with other reindeer *PRNP* alleles. The index case reported by Benestad, Mitchell [2] was of genotype A/A, i.e. an amino acid sequence identical to that found in reindeer susceptible to CWD by oral exposure [23]. Our allele A (Ser225) corresponds to isolate QGAQ of Haley, Rielinger [31] in a Real-Time Quaking-Induced Conversion assay. In that study, the amplification abilities of isolate QGAQ across alleles QGAQ, 225Y (similar to allele B) and SSM (similar to allele E) showed that 225Y and SSM had significant lower amplification rates than QGAQ. These observations are consistent with a scenario in which alleles B (Tyr225) and E (Met2.Ser129.Met169) are less likely to form the initial PrP^{Sc} than allele A (Ser225).

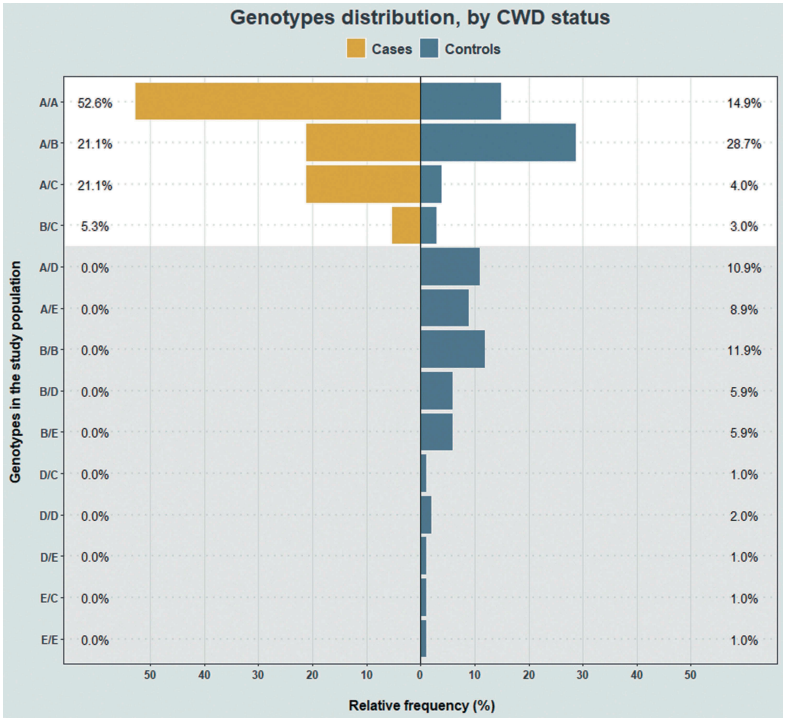

Figure 3. Comparison of *PRNP* genotype frequencies in cases (n = 19) and controls (n = 101). The relative frequencies between cases and controls were statistically different ($P < 0.05$, Fisher's exact test). The plot background in white indicates genotypes found in both groups, and in grey, genotypes only found in controls. The B/B genotype was the most frequent genotype exclusive to controls and selected as the baseline for Firth logistic regression with CWD as an outcome and *PRNP* genotype as a predictor.

Our data support the notion that *PRNP* genetic variation modulates CWD susceptibility rather than conferring complete resistance. This is in agreement with experimental observations of reindeer-developing CWD after intracranial inoculation regardless of *PRNP* genotype [24].

The 3D structure of the C-terminal globular domain of PrP is well conserved in phylogenetically distant organisms and consists of three α-helices of which α2-helix (α2) and α3-helix (α3) are linked by a stabilizing disulphide bond. Moreover, a short anti-parallel β-sheet (β1 and β2 strands) is present, with a loop-structure connecting β2 and the α2 [34]. Interestingly, the structure of the β2-α2 loop, encompassing residues 165–175 (codons 168–178 in reindeer *PRNP*), has been shown to be important for interspecies transmission of prion disease in mice models [35] and the rigidity of the β2-α2 loop, common to cervids [36], at least partly explains the efficient transmission of CWD between cervid species [37]. Recent data suggest that long-range stabilizing interactions between the β2-α2 loop and α3 affect prion propagation [38,39]. For instance, 3D simulations have shown that Ser225Phe, results in a rearrangement allowing a stabilizing hydrogen bond to be established between the β2-α2 loop and α3 that could explain the reduced susceptibility associated with this substitution [30]. A similar effect could be caused by Ser225Tyr in reindeer allele B (Ser225), which could explain the rarity of this allele among the CWD cases.

The identity of the alleles was verified by direct DNA sequencing following cloning and by their occurrence in homozygous animals. With the exception of the novel synonymous substitution (6G>A) and the deletion (249_272del), all *PRNP* alleles were identical to published *Rangifer tarandus* sequences [21,28]. These observations collectively confirm the *PRNP* allele identities as described in our study population.

Comparison of our data with former studies in *Rangifer* spp. show that Gln226 is found constitutively within the genus. Different residues at codon 226 appear to influence the selection and propagation of CWD strains as observed in transgenic [40] and gene-targeted [39] mice bioassays. Mice Gln226 ($GtQ226^{+/+}$) and Glu226 ($GtE226^{+/+}$) respond differently to challenge with the same CWD prion inocula, and the

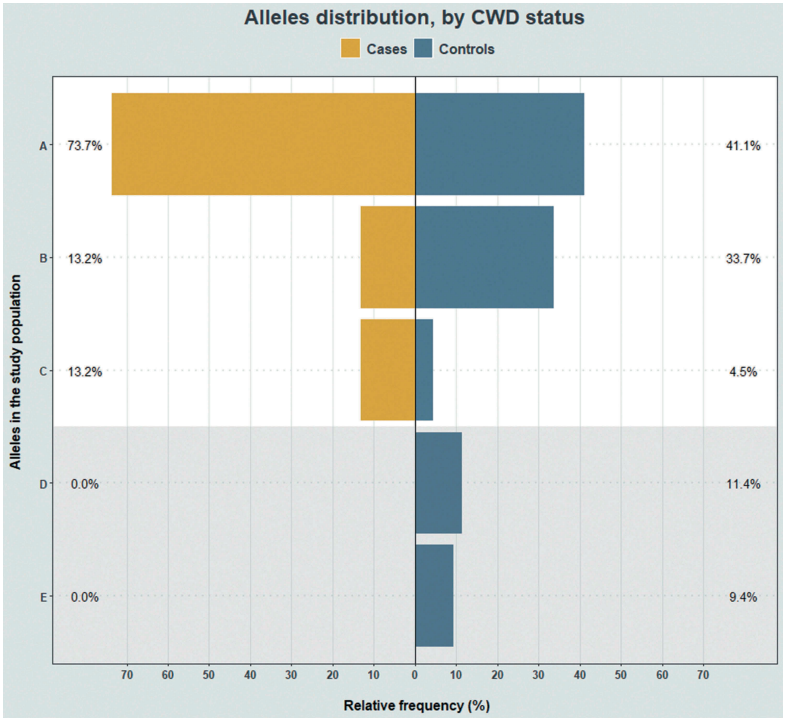

Figure 4. Comparison of *PRNP* alleles' relative frequency by CWD status in wild reindeer from Nordfjella zone 1, Norway. The relative allele frequencies between CWD cases (n= 19) and controls (n = 101) were statistically different ($P < 0.05$, Fisher's exact test). The plot background in white frames genotypes found in cases and controls and in grey, genotypes only found in controls.

Table 2. Analysis of association between numbers of carried copies of either allele A (Ser225) or allele C (deletion) and CWD risk in wild reindeer from Nordfjella zone 1. Regression coefficients and associated statistics from Firth logistic regression. Asterisk indicates a significant value. Abbreviations: S.E. = standard error; C.I. = confidence interval.

Predictor	Regression coefficient	S.E.	95% C.I.		*P*-value	Odds ratio
Constant	−4.04	1.452	−8.89	−2.08	<0.001	-
No copies	Baseline					
Single copy of allele A or C	1.75	1.52	−0.48	6.64	0.144	5.75
Two copies of allele A or C	3.75	1.494	1.63	8.62	<0.001*	42.39

derived prions appear to have distinct conformational properties. Hypothetically, this could denote that diseased deer (Gln226) and elk (Glu226) propagate prions with different strain properties [39]. As appearing in reindeer, Gln226 could select strains that more likely resemble properties of CWD prions in diseased deer (Gln226) rather than in diseased elk (Glu226), which can be important for the risk of interspecies CWD transmission. Demographic factors such as dispersal and population structure impact *PRNP* variation [41]. While the substitutions Ser138Asn (413G>A) [23,28,42] and Asn146 = (438C>T) [28,42] are observed in North American *Rangifer*, but not in the Nordfjella population, the constitutive Ser138 and substitutions at codons 176 and 225 are in accordance with *PRNP* variation previously identified in Norwegian reindeer [21] and absent in North America. The variation patterns in *PRNP* from *Rangifer* spp. as described in different geographic locations encourages further study of their relationship with CWD susceptibility.

The detrimental effects of CWD are progressively being unveiled in North America [43] which implies that the establishment of endemic CWD in Europe could be devastating over time. Further, the culled Nordfjella subpopulation constituted approximately 10% of the remaining wild European tundra reindeer population. Efforts to repopulate Nordfjella zone 1, therefore, have been politically warranted. Any

restocking has to rely on a strategy that is realistic when it comes to preventing reinfection [25].

The description of *PRNP* variation in a naturally infected reindeer population, here presented in association with CWD risk, provides insight into the most susceptible *PRNP* genotypes. The current knowledge does not support genetic resistance towards CWD in cervids. However, knowledge of modulated risk could act as a management tool in both wild and semi-domesticated populations.

Materials and methods

Source population

The Nordfjella wild reindeer management area is located in central Southern Norway (Figure 1). It is divided into zones 1 and 2, which are approximately 2000 km^2 and 1000 km^2 respectively. The two areas are separated primarily by a road, and the presence of tourist cabins and hiking trails also leads to low connectivity between these subpopulations. The initial CWD cases recorded in 2016 were confined to zone 1; thus, the entire subpopulation was culled in a process completed by April 2018 [44].

During 2016–2018, 2424 reindeer were removed from Nordfjella zone 1, which represented our source population. All culled animals were examined for CWD at the Norwegian Veterinary Institute (NVI), which is the national TSE reference laboratory and a World Organization for Animal Health (OIE) CWD reference laboratory. The *medulla oblongata* and retropharyngeal lymph node tissues were tested as a pooled sample for initial screening for the presence of PrP^{Sc} [45]. Pooled samples testing ELISA positive (TeSeE ® SAP ELISA, Bio-Rad) were then re-tested using individual tissues and further verified by Western blot (TeSeE ® Western, Bio-Rad). Any reindeer found PrP^{Sc} positive in at least one tissue (brain or lymph node) was classified as a case. Subsequently, all samples were stored frozen (−20°C).

Study population

A matched case–control study was performed to identify associations between *PRNP* genotypes and CWD risk. The study design included a minimum of four controls (PrP^{Sc} negative) per case (PrP^{Sc} positive) with a match on age category and sex. The rationale being the low prevalence of CWD, and that age and sex could act as confounding variables in the genetic association analysis.

Table 3. Distribution of sex and age category in wild reindeer from Nordfjella zone 1, Norway by CWD status (cases and controls) is presented as absolute and relative frequencies. Young = 15–22 months old < adult.

Sex	Age category	Cases (*n*= 19)	Controls (*n* = 101)
Male	Young	1 (5.3%)	2 (2.0%)
	Adult	12 (63.2%)	68 (67.3%)
Female	Young	0 (0.0%)	3 (3.0%)
	Adult	6 (31.6%)	28 (27.7%)

For all cases and controls, the sex and the age categories were based on autopsy (cases) or information from the hunters (controls). The study population included all CWD cases with matched controls in the source population.

We analysed *PRNP* variation in a total of 120 wild reindeer: 19 cases and 101 controls. The cases were sampled between March 2016 and April 2018. The controls were randomly selected from a selection pool consisting of stored samples from Nordfjella zone 1 collected between March 2016 and October 2017. The matching on age category and sex for cases and controls was close to equal (Table 3).

PRNP sequencing

Genomic DNA was extracted from brain tissues with DNeasy® Blood and Tissue kit (Qiagen, Oslo, Norway). The ORF of *PRNP* was amplified using PCR primers Ce19_F (5′-ATTTTGCAGATAAGTCATC-3′) and Ce778_R (5′-AGAAGATAATGAAAACAGGAAG-3′) designed by O'Rourke, Spraker [46]. The PCR reaction contained 2 µl genomic DNA as template, 2 µl dNTPs mix (4 x 2.5 mM) (WVR, Radnor, PA, USA), 0.6µl forward and reverse primers (10 pmol) (Eurofins genomics, Luxembourg, Luxembourg), 2 µl Key Buffer (15 mM $MgCl_2$) (VWR, Radnor, PA, USA), 0.1 µl Taq DNA polymerase (5 U/µl) (VWR Radnor, PA, USA) and purified water to a final reaction volume of 20 µl. The PCR amplification started with an initial cycle at 95°C for 2 min, followed by 36 cycles at 95°C for 30 s, 51°C for 30 s and 72°C for 45 s, then a final cycle at 72°C for 10 min.

PCR products were visualized in a 1.5% agarose gel and purified by illustra™ ExoProStar™ 1-Step (GE Healthcare, Uppsala, Sweden). The sequencing reaction was performed using the initial PCR primers and a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer recommendations. Finally, sequence data for both strands were generated using a 3500xL Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

Some samples required an additional PCR sequencing reaction for optimal visualization of the initial sequence in the ORF. For this purpose, we used primer set Ce19_F and Prp-157_R (5′-ACTTCCCTGTCCCGGGTAT-3′, in-house). A group of samples were additionally tested for the presence of the *PRNP* pseudogene (*PRNPψ*), with primers 369/224 [46] with visualization of the PCR product on a 1.5% agarose gel utilizing PCR parameters as given above.

Sequences were aligned with SeqScape v3.0 (Applied Biosystems) and edited in MEGA7 version 7.0.26 [47]. The Human Genome Variation Society nomenclature guidelines (version 15.11) were used to describe the sequence variants [48] and the nomenclature was checked with Mutalyzer 2.0.28 [49]. The description of *PRNP* variant positions and alleles is based on reference sequences DQ154293.1 for nucleotides and AAZ81474.1 for amino acids. Sequences were treated as unphased genotypes to infer two alleles per individual by a conservative best-fit approach, which considered the minimum possible number of alleles together with pairwise Linkage disequilibrium (LD) analysis between variant positions.

Cloning

PRNP coding sequences were amplified (standard conditions) from the genomic DNA of three heterozygous animals with PCR primers modified to introduce *EcoRI* and *NotI* restriction sites at the ends of the amplicon (Ce19m_F: 5′-AGTCGAATTCATTTTGCAGATAAGT CATC-3′ and Ce778m_R: 5′-TGACGCGGCCGCAGA AGATAATGAAAACAGGAAG-3′). The amplicons were then cloned into the pCI-neo Mammalian Expression Vector (Promega, Madison, WI, USA) using standard cloning techniques. Selected clones were sequenced as described above to independently confirm each allele.

Cell culture and transfection

The human neuroblastoma cell line SH-SY5Y (RRID: CVCL_0019) (Sigma-Aldrich, St. Louis, MO, USA) was cultured as previously described [50].

Plasmid constructs encoding either allele A (Ser225) (SH-SY5Y rePrP^{wt}) or allele C (deletion) (SH-SY5Y rePrP^{del}) were transiently transfected into the SH-SY5Y cells using jetPRIME® (Polyplus-transfection® SA, Illkirch, France) following the manufacturer's protocol.

Western blot

Cells were lysed with homogenizing buffer (Tris HCl 50 uM, NaCl 150 mM, EDTA 1 mM, DOC 0.25%, NP40 1%) supplemented with cOmplete™ protease inhibitor cocktail (Roche, Basel, Switzerland). The protein concentration was determined with the Protein Assay kit I (Bio-Rad, Hercules, CA, USA). For each sample, 20 µg protein was deglycosylated with PNGase-F (New England BioLabs Inc) in accordance with the manufacturer's guidelines, and 20 µg protein were analysed untreated. The samples were denatured using sodium dodecyl sulphate (SDS) loading buffer (Invitrogen) and Sample Reducing Agent (Thermo Fisher Scientific) before separation by polyacrylamide gel electrophoresis (PAGE) on a 12% Criterion™ XT Bis-Tris gel (Bio-Rad). Proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (GE Healthcare, Chicago, IL, USA) and the membrane blocked with 5% non-fat dried milk in TBS – Tween. The membrane was then incubated with primary antibody (P4 mouse anti PrPC; Ridascreen Biopharm AG, Darmstadt, Germany) 1:1000, and secondary antibody (anti-mouse IgG conjugated to alkaline phosphatase; Invitrogen) 1:1000. Detection with EFC™ substrate (GE Healthcare) was performed on a Typhoon 9200 imager (Amersham Biosciences, Sunnyvale, CA, USA).

Statistical analysis

The statistical analyses were executed using R version 3.5.2 [51] and RStudio version 1.1.456 [52] using *genetics* and *logistf* packages. Results were plotted using *dplyr* and *ggplot2* packages.

Categorical variables were summarized into absolute (counts) and relative (percentages) frequencies for cases and controls. Contingency tables were analysed by Fisher's exact test (or Chi-square test if suitable). Tests were two-sided and a *P*-values <0.05 were interpreted as significant.

HWE fitness was tested in the control group for genotypes at each variant position within the *PRNP* sequence; a *P*-value <0.05 was interpreted as a departure from HWE. Linkage disequilibrium between variant position pairs was measured by D' and r^2. After interpretation of the *PRNP* alleles, phased genotypes of the *PRNP* sequence were evaluated for HWE fitness in the controls. *PRNP* allele and genotype associations with CWD status were tested by Fisher's exact test.

The Firth method reduces small-sample bias in the logistic model due to the small number of cases. Thus, Firth logistic regression was used to investigate the relationship between CWD outcome and *PRNP* genotype. The CWD risk was assessed in terms of the odds ratio, which resulted from the exponentiation of the regression coefficients.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Haley NJ, Hoover EA. Chronic wasting disease of cervids: current knowledge and future perspectives. Annu Rev Anim Biosci. 2015;3(1):305–325.
[2] Benestad SL, Mitchell G, Simmons M, et al. First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer. Vet Res. 2016 Sep 15;47(1):88.
[3] Pirisinu L, Tran L, Chiappini B, et al. Novel type of chronic wasting disease detected in moose (Alces alces), Norway. Emerg Infect Dis. 2018 Dec;24 (12):2210–2218.
[4] Chronic Wasting Disease (CWD) found in a captive reindeer in Northern Illinois [Internet]. Springfield, IL; 2018 June 8;[cited 2019 Aug 02]. Available from: https://www.agr.state.il.us/press.php?fn=chronic-wasting-disease-cwd-found-in-a–captive-reindeer-in-northern-il-2018
[5] Vikøren T, Våge J, Madslien KI, et al. First detection of chronic wasting disease in a wild red deer (Cervus elaphus) in Europe. J Wildl Dis. 2019 Mar 28;55:970.
[6] Schwabenlander MD, Culhane MR, Hall SM, et al. A case of chronic wasting disease in a captive red deer (Cervus elaphus). J Vet Diagn Invest. 2013;25 (5):573–576.
[7] PRION. 2019 emerging concepts. Prion: Taylor & Francis; 2019 May 15.
[8] Bolton DC, McKinley MP, Prusiner SB. Identification of a protein that purifies with the scrapie prion. Science (New York, NY). 1982 Dec 24;218(4579):1309–1311.
[9] Prusiner SB, Bolton DC, Groth DF, et al. Further purification and characterization of scrapie prions. Biochemistry. 1982 Dec 21;21(26):6942–6950.
[10] Brandner S, Raeber A, Sailer A, et al. Normal host prion protein (PrPC) is required for scrapie spread within the central nervous system. Proc Natl Acad Sci U S A. 1996 Nov 12;93(23):13148–13151.
[11] Miller MW, Williams ES, Hobbs NT, et al. Environmental sources of prion transmission in mule deer. Emerg Infect Dis. 2004 Jun;10(6):1003–1006.
[12] Zabel M, Ortega A. The ecology of Prions. Microbiol Mol Biol Rev. 2017 Sep;81:3.
[13] Johnson CJ, Pedersen JA, Chappell RJ, et al. Oral transmissibility of prion disease is enhanced by binding to soil particles. PLoS Pathog. 2007 Jul;3(7):e93.
[14] Morales R, Abid K, Soto C. The prion strain phenomenon: molecular basis and unprecedented features. Biochim Biophys Acta (BBA) - Mol Basis Dis. 2007 Jun 01;1772(6):681–691.
[15] O'Rourke KI, Spraker TR, Zhuang D, et al. Elk with a long incubation prion disease phenotype have a unique PrPd profile. Neuroreport. 2007 Dec 3;18 (18):1935–1938.
[16] Perucchini M, Griffin K, Miller MW, et al. PrP genotypes of free-ranging wapiti (Cervus elaphus nelsoni) with chronic wasting disease. J Gen Virol. 2008;89 (5):1324–1328.
[17] Fox KA, Jewell JE, Williams ES, et al. Patterns of PrPCWD accumulation during the course of chronic wasting disease infection in orally inoculated mule deer (Odocoileus hemionus). J Gen Virol. 2006;87(11):3451–3461.
[18] Johnson CJ, Herbst A, Duque-Velasquez C, et al. Prion protein polymorphisms affect chronic wasting disease progression. Plos One. 2011;6(3):e17450.
[19] Johnson C, Johnson J, Vanderloo JP, et al. Prion protein polymorphisms in white-tailed deer influence susceptibility to chronic wasting disease. J Gen Virol. 2006;87(7):2109–2114.
[20] Robinson SJ, Samuel MD, O'Rourke KI, et al. The role of genetics in chronic wasting disease of North American cervids. Prion. 2012 Apr-Jun;6(2):153–162.
[21] Wik L, Mikko S, Klingeborn M, et al. 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. 2012 Jul 1;6(3):256–260.
[22] Robinson AL, Williamson H, Güere ME, et al. Variation in the prion protein gene (PRNP) sequence of wild deer in Great Britain and mainland Europe. Vet Res. 2019 Jul 31;50(1):59.
[23] Mitchell GB, Sigurdson CJ, O'Rourke KI, et al. Experimental oral transmission of chronic wasting disease to reindeer (Rangifer tarandus tarandus). Plos One. 2012;7(6):e39055.
[24] Moore SJ, Kunkle R, West Greenlee MH, et al. Horizontal transmission of chronic wasting disease in reindeer. Emerg Infect Dis J. 2016;22(12):2142.
[25] VKM. CWD in Norway – a state of emergency for the future of cervids (Phase II). In: Opinion of the panel on biological hazards of the Norwegian Scientific Committee for food safety. Oslo: Vitenskapskomiteen for mattrygghet (VKM); 2017:1–125.

[26] Grendstad EE (e.f.), Ormsettrø SI. Tiltak mot CWD/skrantesjuke i Nordfjella villreinområde. editor. 16/474-. In: matdepartementet DKL-o. Oslo: Regjeringen; 2017.
[27] The Norwegian Nature Inspectorate. Chronic wasting disease: all known animals in Nordfjella dispatched. Oslo: The Ministry of Agriculture and Food's website; 2018.
[28] Happ GM, Huson HJ, Beckmen KB, et al. PRION PROTEIN GENES IN CARIBOU FROM ALASKA. J Wildl Dis. 2007;43(2):224–228.
[29] Jewell JE, Conner MM, Wolfe LL, et al. Low frequency of PrP genotype 225SF among free-ranging mule deer (Odocoileus hemionus) with chronic wasting disease. J Gen Virol. 2005;86(8):2127–2134.
[30] Angers R, Christiansen J, Nalls AV, et al. Structural effects of PrP polymorphisms on intra- and interspecies prion transmission. Proc Nat Acad Sci. 2014 Jul 29;111(30):11169–11174.
[31] Haley NJ, Rielinger R, Davenport KA, et al. Estimating chronic wasting disease susceptibility in cervids using real-time quaking-induced conversion. J Gen Virol. 2017 Nov;98(11):2882–2892.
[32] Vaccari G, D'Agostino C, Nonno R, et al. Prion protein alleles showing a protective effect on the susceptibility of sheep to scrapie and bovine spongiform encephalopathy. J Virol. 2007;81(13):7306–7309.
[33] Vaccari G, Scavia G, Sala M, et al. Protective effect of the AT137RQ and ARQK176 PrP allele against classical scrapie in Sarda breed sheep. Vet Res. 2009;40(3):19.
[34] Calzolai L, Lysek DA, Pérez DR, et al. Prion protein NMR structures of chickens, turtles, and frogs. Proc Natl Acad Sci U S A. 2005;102(3):651–655.
[35] Sigurdson CJ, Nilsson KPR, Hornemann S, et al. A molecular switch controls interspecies prion disease transmission in mice. J Clin Invest. 2010 Jul 01;120(7):2590–2599.
[36] Gossert AD, Bonjour S, Lysek DA, et al. Prion protein NMR structures of elk and of mouse/elk hybrids. Proc Natl Acad Sci U S A. 2005;102(3):646–650.
[37] Pérez DR, Damberger FF, Wüthrich K. Horse prion protein NMR Structure and comparisons with related variants of the mouse prion protein. J Mol Biol. 2010 Jul 09;400(2):121–128.
[38] Moreno JA, Telling GC. Molecular mechanisms of chronic wasting disease prion propagation. Cold Spring Harb Perspect Med. 2018 June 1;8(6):a024448.
[39] Bian J, Christiansen JR, Moreno JA, et al. Primary structural differences at residue 226 of deer and elk PrP dictate selection of distinct CWD prion strains in gene-targeted mice. Proc Natl Acad Sci. 2019;116(25):12478–12487. doi: 10.1073/pnas.1903947116.
[40] Angers RC, Seward TS, Napier D, et al. Chronic wasting disease prions in elk antler velvet. Emerg Infect Dis. 2009 May;15(5):696–703.
[41] Miller WL, Walter WD. Spatial heterogeneity of prion gene polymorphisms in an area recently infected by chronic wasting disease. Prion. 2019 Jan;13(1):65–76.
[42] Cheng YC, Musiani M, Cavedon M, et al. High prevalence of prion protein genotype associated with resistance to chronic wasting disease in one Alberta woodland caribou population. Prion. 2017 Mar 04;11(2):136–142.
[43] DeVivo MT, Edmunds DR, Kauffman MJ, et al. Endemic chronic wasting disease causes mule deer population decline in Wyoming. PloS One. 2017;12(10):e0186512.
[44] Mysterud A, Rolandsen CM. A reindeer cull to prevent chronic wasting disease in Europe. Nat Ecol Evol. 2018 Sep;2(9):1343–1345.
[45] Viljugrein H, Hopp P, Benestad SL, et al. A method that accounts for differential detectability in mixed samples of long-term infections with applications to the case of chronic wasting disease in cervids. Methods in Ecology and Evolution. 2019;10(1):134–145. doi: 10.1111/2041-210X.13088
[46] O'Rourke KI, Spraker TR, Hamburg LK, et al. 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. 2004;85(5):1339–1346.
[47] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016 Jul;33(7):1870–1874.
[48] den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS recommendations for the description of sequence variants: 2016 update. Hum Mutat. 2016 Jun;37(6):564–569.
[49] Wildeman M, van Ophuizen E, den Dunnen JT, et al. Improving sequence variant descriptions in mutation databases and literature using the Mutalyzer sequence variation nomenclature checker. Hum Mutat. 2008;29(1):6–13.
[50] Malachin G, Reiten MR, Salvesen Ø, et al. Loss of prion protein induces a primed state of type I interferon-responsive genes. Plos One. 2017;12(6):e0179881.
[51] R Core Team. R: A language and environment for statistical computing. 3.5.2. Vienna, Austria; 2018.
[52] RStudio Team. RStudio: integrated development environment for R. 1.1.456. Boston, MA: R Foundation for Statistical Computing; 2015. Available from: https://www.r-bloggers.com/its-easy-to-cite-and-reference-r/

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Supplementary data

Supplementary table 1. Variant positions in *PRNP* coding sequence from wild reindeer from Nordfjella zone 1. Variant descriptions are in accordance to the HGVS nomenclature definition, using the three-letter amino acid symbol. DNA and protein level descriptions are reported in comparison to reference sequences DQ154293.1 (nucleotide) and AAZ81474.1 (protein). Frequency analysis is based on variance at the nucleotide level.

Abbreviations: *PRNP*= prion protein gene; HGVS = Human Genome Variant Society; DNA = deoxyribonucleic acid.

DNA variation	**Predicted protein variation**	**Minor Allele Frequency**
4G>A	Val2Met	0.08
6G>A	Val2=	0.02
249_272del	Trp84_Gly91del	0.06
385G>A	Gly129Ser	0.08
505G>A	Val169Met	0.08
526A>G	Asn176Asp	0.10
674C>A	Ser225Tyr	0.30

Supplementary table 2. *PRNP* alleles in wild reindeer from Nordfjella zone 1. The alleles represent the DNA arrangement within *PRNP* coding sequence predicted to encode unique prion proteins. Alleles were constructed by phasing non-synonymous variant positions from the study population. Variant descriptions are in accordance to the HGVS nomenclature definition, using the three-letter amino acid symbol. DNA and protein level descriptions are reported in comparison to reference sequences DQ154293.1 (nucleotide) and AAZ81474.1 (protein).

Abbreviations: *PRNP*= prion protein gene; HGVS = Human Genome Variant Society; DNA = deoxyribonucleic acid.

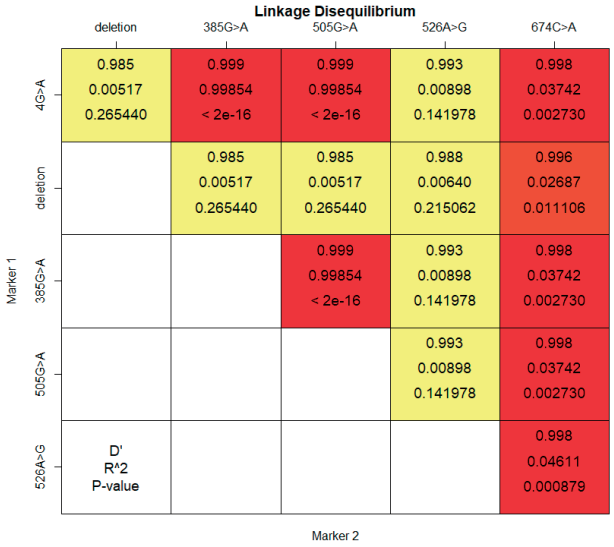
Allele	**DNA variant**	**Predicted protein variant**
A	DQ154293.1	AAZ81474.1
B	DQ154293.1:c.[674C>A]	AAZ81474.1:p.[(Ser225Tyr)]
C	DQ154293.1:c.[249_272del]	AAZ81474.1:p.[(Trp84_Gly91del)]
D	DQ154293.1:c.[526A>G]	AAZ81474.1:p.[(Asn176Asp)]
E	DQ154293.1:c.[4G>A;385G>A;505G>A]	AAZ81474.1:p.[(Val2Met;Gly129Ser;Val169Met)]
A2	DQ154293.1:c.[6G>A]	AAZ81474.1:p.[(Val2=)]

Supplementary table 3. Analysis of association between *PRNP* genotypes and CWD risk in wild reindeer from Nordfjella zone 1 culled between March 2016 and April 2018. Regression coefficients and associated statistics from Firth logistic regression. B/B genotype served as the baseline for being the most frequent genotype restricted to the controls. Asterisk indicates a significant value.

Abbreviations: *PRNP* = prion protein gene; CWD = chronic wasting disease; S.E. = standard error; C.I. = confidence interval.

Predictor	**Regression coefficient**	**S.E.**	**95% CI**		***P*-value**	**Odds ratio**
Constant	-3.219	1.501	-8.072	-1.193	0	-
B/B	Baseline					
A/A	2.829	1.555	0.613	7.722	0.008*	16.94
A/B	1.339	1.587	-1.015	6.253	0.308	3.81
A/C	3.219	1.659	0.715	8.182	0.009*	25.00
A/D	0.083	2.128	-5.175	5.342	0.967	1.09
A/E	0.274	2.143	-4.988	5.537	0.893	1.32
B/C	2.372	1.856	-0.764	7.449	0.137	10.71
B/D	0.654	2.183	-4.62	5.929	0.752	1.92
B/E	0.654	2.183	-4.62	5.929	0.752	1.92
D/C	2.12	2.754	-3.266	7.543	0.354	8.33
D/D	1.609	2.419	-3.72	6.952	0.463	5.00

Predictor	Regression coefficient	S.E.	95% CI		*P*-value	Odds ratio
D/E	2.12	2.754	-3.266	7.543	0.354	8.33
E/C	2.12	2.754	-3.266	7.543	0.354	8.33
E/E	2.12	2.754	-3.266	7.543	0.354	8.33



Supplementary figure 1. Pairwise LD analysis between non-synonymous variant positions (nucleotide) in *PRNP* coding sequence. Each frame summarizes LD between marker 1 and marker 2 measured by D', R^2 and *P*-value (order shown in the lower left frame). Color-shading based on *P*-value where red indicates a high significance. Abbreviations: LD = linkage disequilibrium; *PRNP* = prion protein gene; D' = scaled disequilibrium statistic; R^2 = mean squared correlation coefficient

Article III

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VETERINARY RESEARCH

RESEARCH ARTICLE

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Variation in the prion protein gene (*PRNP*) sequence of wild deer in Great Britain and mainland Europe

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Abstract

Susceptibility to prion diseases is largely determined by the sequence of the prion protein gene (*PRNP*), which encodes the prion protein (PrP). The recent emergence of chronic wasting disease (CWD) in Europe has highlighted the need to investigate *PRNP* gene diversity in European deer species, to better predict their susceptibility to CWD. Here we report a large genetic survey of six British deer species, including red (*Cervus elaphus*), sika (*Cervus nippon*), roe (*Capreolus capreolus*), fallow (*Dama dama*), muntjac (*Muntiacus reevesii*), and Chinese water deer (*Hydropotes inermis*), which establishes *PRNP* haplotype and genotype frequencies. Two smaller data sets from red deer in Norway and the Czech Republic are also included for comparison. Overall red deer show the most *PRNP* variation, with non-synonymous/coding polymorphisms at codons 98, 168, 226 and 247, which vary markedly in frequency between different regions. Polymorphisms P168S and I247L were only found in Scottish and Czech populations, respectively. T98A was found in all populations except Norway and the south of England. Significant regional differences in genotype frequencies were observed within both British and European red deer populations. Other deer species showed less variation, particularly roe and fallow deer, in which identical *PRNP* gene sequences were found in all individuals analysed. Based on comparison with *PRNP* sequences of North American cervids affected by CWD and limited experimental challenge data, these results suggest that a high proportion of wild deer in Great Britain may be susceptible to CWD.

Introduction

Chronic wasting disease (CWD) is a prion disease of cervid species that is widespread in North America and has recently emerged in Europe. It belongs to a family of diseases termed transmissible spongiform encephalopathies (TSEs), which cause progressive and invariably fatal neurodegenerative disorders in humans and animals. CWD was first described in the 1960s as a wasting syndrome of captive mule deer (*Odocoileus hemionus hemionus*) and black-tailed deer (*Odocoileus hemionus columbianus*) in Colorado wildlife facilities [1]. It has since been identified in North America in wapiti (elk, *Cervus canadensis*) white-tailed deer (*Odocoileus virginianus*), moose (*Alces alces*) and a single captive red deer (*Cervus elaphus*) [2, 3]. It is currently the only animal prion disease recognised in both captive and wild populations, and efficient transmission has led to spread of the disease across North America. At the time of writing, CWD is present in at least 25 US states and three Canadian provinces [4], as well in South Korea, introduced via importation of infected wapiti [5].

Member States of the European Union (EU) and Norway were believed to be free of CWD based on a survey conducted between 2006 and 2009 on 3274 farmed and 10 049 wild deer [6], with the vast majority being red deer (10 110). However, the first European case of CWD, and the first natural case in a reindeer (*Rangifer tarandus*) was identified in Norway in March 2016 [7].

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Subsequent culling of the affected reindeer population resulted in detection of another 18 infected animals, and increased surveillance in Scandinavia has identified further "atypical" cases in three moose and a single red deer in separated locations in Norway, and individual moose in Finland and Sweden. Although the origins of CWD in Scandinavia are uncertain, the wide geographic distribution of cases raises concern that disease may have been present and undetected for some time [8–11].

The development of prion diseases is associated with misfolding of the prion protein (PrP^{C}) into a protease-resistant form (PrP^{Sc}). The sequence of the open reading frame (ORF) of the gene encoding the prion protein (*PRNP)* is strongly associated with susceptibility to prion diseases. This association has been exploited in breeding programmes such as the National Scrapie Plan in the UK, which selectively bred for disease resistant *PRNP* genotypes to reduce the incidence of classical scrapie in sheep [12, 13]. Cervid *PRNP* genetics have been widely studied in North America, where both natural and experimental infection of deer species has allowed for the identification of *PRNP* polymorphisms that are associated with reduced incidence of disease and/or slower disease progression. The first identified was M132L in wapiti, which encodes a methionine to leucine change at codon 132. Genotype surveys of CWD affected deer populations have produced conflicting evidence regarding the association of codon 132 variation with incidence of disease [14, 15], but CWD challenges of deer [16, 17] and transgenic mice [18] suggest that the 132L variant reduces susceptibility to infection. Other important *PRNP* polymorphisms associated with CWD susceptibility include S225F in mule deer, [19–22], Q95H, G96S and A116G in white-tailed deer [23–28]. Although many of these *PRNP* polymorphisms have been associated with reduced susceptibility to CWD, none of them appear to confer complete resistance.

Experimental transmissions of CWD provide some evidence of susceptibility in deer species found in Europe. CWD has been transmitted orally to red deer [29], reindeer [30] and Reeves' muntjac deer [31, 32]. Fallow deer have been infected via intracerebral inoculation [33], but failed to become infected when co-grazed with infected mule deer [34]. However, due to small sample sizes in all these studies, it is difficult to draw associations between susceptibility to experimental CWD infection and host *PRNP* genotype. It is also not known if the *PRNP* genotypes of the experimental animals used in these North American studies are representative of the genetics of native wild European deer populations.

The extent of *PRNP* sequence variation in European deer species has not previously been studied in detail, and this genetic information will be critically important in estimating potential susceptibility of these populations to emerging CWD and informing risk assessment and control/surveillance strategies. Novel *PRNP* variants (not seen in North American cervids) that show evidence of association with reduced susceptibility or resistance to CWD have the potential to be used in breeding programmes for captive and farmed deer. The primary aims of this study were (i) to perform a comprehensive survey of the protein-coding sequence of the *PRNP* gene in the six major free-ranging or wild species of deer found in Great Britain (GB) and (ii) to compare the *PRNP* genotype distributions in British and European populations of red deer, as red deer are one of the most numerous wild species in both GB and mainland Europe, and are also an economically important game species due to revenue generated via hunting, tourism and venison production [35].

Materials and methods

Samples from British deer species

A total of 1003 samples collected from wild deer in GB (England, Scotland and Wales) were analysed. Archived DNA samples were provided by JP, SPE and SLS for red deer, sika deer, and red/sika hybrids, and by KB for roe deer. The procedures for sample collection and genomic DNA preparation for these archives have been described previously [36–38]. Additional samples were collected by stalkers in the British Deer Society to give representation of geographical areas and species not covered by the existing archives. These included samples collected during routine culling, and samples collected from culled deer at a meat processing facility. Samples consisted of small sections of ear tips, which were preserved in 20% DMSO/saturated NaCl solution during transportation/storage [39] For each sample, further information including the sex, estimated age, and postcode/grid coordinates of the location of the animal, were recorded. The distribution of sampling sites for each deer species are shown in Figure 1.

In total, samples from 480 red deer were analysed, of which 388 were from the archived collection, and 92 samples were collected by BDS deer stalkers. Samples from 83 sika deer were analysed, 73 samples were archived DNA, and 10 stalker collected samples from the South of England. All samples collected by members of the BDS were identified by animal phenotype only, whereas archived DNA samples of red and sika deer were selected both on phenotypical appearance and microsatellite genotyping data [38, 40], whereby a Q value of $Q=1$ indicated a pure red deer, $Q=0$ indicated a pure sika deer. Nine sika-red hybrids were also included in this study and were classified as having $0.091 \leq Q \leq 0.772$.

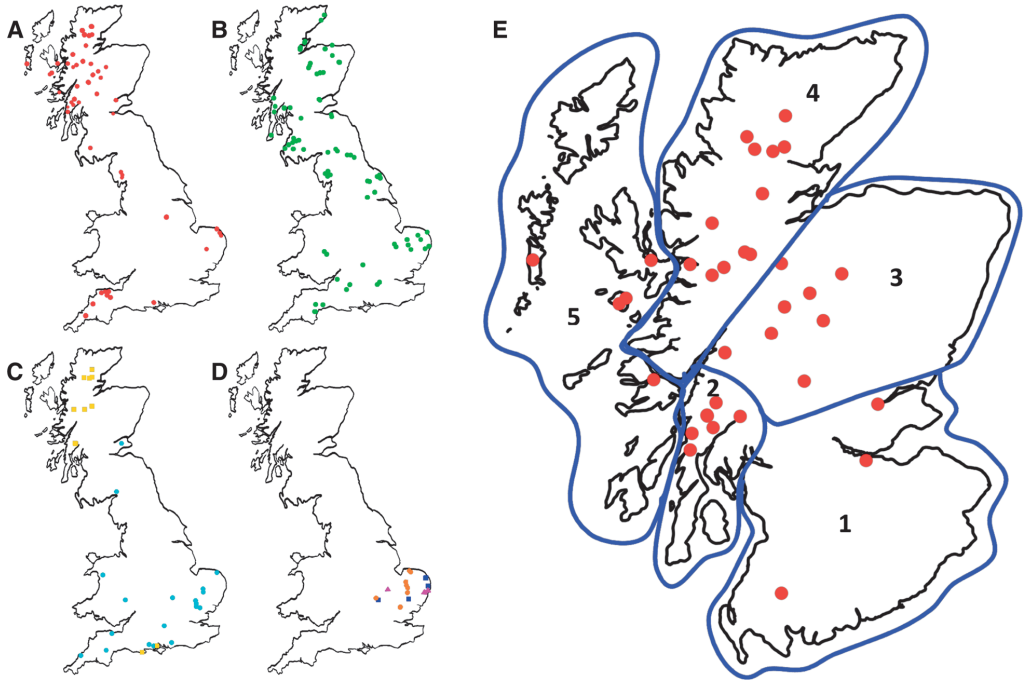

Figure 1 Maps to show the sampling locations of deer samples from Great Britain. Each point represents one sampling location with ≤ 28 animals sampled. **A** Red deer (red circles), **B** roe deer (green circles), **C** sika (yellow squares) and fallow (blue circles), **D** muntjac (orange circles) and Chinese water deer (dark blue squares), or both muntjac and Chinese water deer (pink triangles). **E** Red deer samples in Scotland separated into regions: South Scotland (1), Argyll (2), Central Scotland (3), North Highlands (4) and Hebrides (5)

For analysis red deer populations were separated into the following regions: Southern England (mainly Exmoor National Park) ($n=78$), Northern England (Lake District) ($n=32$), Southern Scotland ($n=23$), Argyll ($n=47$), Central Highlands ($n=142$), Northern Highlands ($n=114$) and Hebrides ($n=44$) (Figure 1).

A total of 297 samples from roe deer were analysed, 171 from a DNA archive [37], and 126 collected by BDS deer stalkers. Samples from 66 fallow deer and 41 muntjac deer were collected by BDS deer stalkers. A total of 27 Chinese Water deer samples were analysed, 3 from a DNA archive [41] and 24 supplied by BDS deer stalkers.

Samples from other European red deer

Genotype analysis was also performed on 50 wild red deer collected from six counties in Norway, and 66 archived DNA samples from wild red deer ($n=46$) and wild sika ($n=20$) collected in five regions of the Czech Republic [42, 43]. In central Europe, 2 distinct lineages of red deer are present. Of the 46 Czech red deer, 24 were of Western lineage, and 22 of Eastern lineage.

Extraction and purification of genomic DNA

Genomic DNA was extracted from small sections of ear-tip tissue, digested with proteinase K (PK) at 37 °C overnight, followed by standard phenol/chloroform purification, ethanol precipitation and re-suspension in 1XTE buffer, as described previously [44]. Norwegian samples were prepared from brain tissue using DNeasy® Blood & Tissue Kit (Qiagen, Oslo, Norway) according to manufacturer's instructions.

PRNP gene amplification and sequencing: British and Czech samples

The ORF of cervid *PRNP* (771 bp) was amplified by PCR using AmpliTaq Gold 360 master mix (Thermo Fisher Scientific) and primer pairs −143d (ATGGAATGTGAA GAACATTTATGACCTA) or −213d (AGGTCAACT TTGTCCTTGGAGGAG) with +139u (TAAGCGCCA AGGGTATTAGCAT) or AR2 (GCAAGAAATGAG ACACCACCAC) for British and Czech red, roe and sika deer, −213d and AR3 (ACCACTACAGGGCTGCAG GTA) for fallow and Chinese water deer, and −213d and

AR2 (GCAAGAAATGAGACACCACCAC) for muntjac deer. PCR conditions were 95 °C for 5 min followed by 40 cycles of 95 °C for 30 s, 61 °C for 30 s and 72 °C for 1 min, and a final 72 °C for 10 min. PCR fragments were sequenced by sanger sequencing using primer +70u (GCTGCAGGTAGATACTCCCTC) and BigDye® reagents (Life Technologies, Paisley, UK) as recommended by the manufacturer. Sequence data were analysed using Chromas and DNAStar Lasergene version 14.

PRNP gene amplification and sequencing: Norwegian samples

The ORF was amplified using PCR primers Ce19_F (ATT TTGCAGATAAGTCATC) and Ce 778_R (AGAAGA TAATGAAAACAGGAAG) [14]. PCR conditions were 95 °C for 2 min, followed by 36 cycles at 95 °C for 30 s, 51 °C for 30 s and 72 °C for 45 s, then a final cycle at 72 °C for 10 min. PCR fragments were directly sequenced using Ce19_F and Ce 778_R and BigDye® reagents (Life Technologies, Paisley, UK) as recommended by the manufacturer. Sequence data were analysed using Seqscape v3.0 software, Sequence Scanner v2.0 (Applied Biosystems) and MEGA7 version 7.0.26 [45].

Statistical analysis

The Chi square test was used to compare haplotype frequencies between different regions and countries.

Accession numbers

Sequences were deposited in Genbank with the following accession numbers: *Capreolus capreolus* MK103016; *Dama dama* MK103017; *Cervus nippon* MK103018, MK103019; *Muntiacus reeveesi* MK103020–MK103023; *Hydropotes inermis* MK103024–MK103026; *Cervus elaphus* with 247L MK103027.

Results

The ORF encoded by exon 3 of the *PRNP* gene is highly conserved among cervid species, and non-synonymous polymorphisms identified in individual species are usually reported as variations from a consensus sequence [25]. Following this convention, the amino acid substitutions resulting from *PRNP* sequence polymorphisms identified in the deer species examined in our survey are summarized in Table 1.

British red deer

Four single nucleotide polymorphisms (SNPs) were identified in 480 British red deer samples: a synonymous SNP at codon 136 (t/c, nucleotide position 408), and three non-synonymous polymorphisms at codons 98 (a/g, nucleotide position 292), 168 (c/t, nucleotide position 502) and 226 (g/c, nucleotide position 676) giving rise to amino acid changes threonine to alanine (T98A), proline to serine (P168S) and glutamine to glutamic acid (Q226E), respectively. Linkage was found between positions 408 and 676 (codons 136 and 226), such that the haplotypes were either 408t-676c or 408c-676g. The SNPs resulting in substitutions at positions 98 and 168 occurred exclusively on a Q226 background and the following four haplotypes were inferred: T_{98}-P_{168}-E_{226} (TPE), T_{98}-P_{168}-Q_{226} (TPQ), A_{98}-P_{168}-Q_{226} (APQ) and A_{98}-S_{168}-Q_{226} (ASQ).

Norwegian and Czech red deer

Only the TPE and TPQ haplotypes were identified in Norwegian red deer. The synonymous codon 136 polymorphism was observed in the same haplotype linkage as described above. Czech red deer had TPE, TPQ and APQ haplotypes, as well as a novel non-synonymous SNP at codon 247 (a/c nucleotide position 739) leading to a conservative isoleucine to leucine substitution which occurred in linkage with APQ (APQ-L_{247}).

British sika, Czech sika and sika-red deer hybrids

Scottish pure sika ($n=73$), Czech sika ($n=20$), English sika ($n=8/10$) were all homozygous for the TPQ haplotype. Both British and Czech sika had a synonymous polymorphism at codon 133 (g/c, position 399) at frequencies of 0.08% and 0.1% respectively. Of the nine genetically determined sika-red hybrids from Argyll ($0.091 \leq Q \leq 0.772$) three were homozygous TPQ/TPQ, four were heterozygous TPE/TPQ, and two were heterozygous for the APQ haplotype (APQ/TPQ and APQ/TPE). The two animals heterozygous for the APQ haplotype were characterised as red deer-like ($Q=0.71$, 0.74), as were two of four TPE/TPQ heterozygotes ($Q=0.77$, 0.62). Of the 10 English animals identified as sika by phenotype only, two were TPE/TPQ heterozygous and were therefore possibly sika-like hybrids.

Roe and fallow deer

The *PRNP* ORF of British roe deer ($n=297$) all encoded the haplotype described above as TPQ. Roe deer were monomorphic in *PRNP* sequence, with no variation in nucleotide sequence in any of the animals analysed. Likewise, fallow deer ($n=66$) showed no variation in *PRNP* sequence and encoded the TPE haplotype with an additional amino acid substitution of asparagine for serine at codon 138 (TPE-N_{138}).

Table 1 Amino acid variation within the ORF of cervid *PRNP* in the surveyed deer species

	***PRNP* codon number**					
	98	**100**	**138**	**168**	**226**	**247**
Consensus sequence	T	S	S	P	Q	I
Red *Cervus elaphus*						
TPQ	–	–	–	–	–	–
TPE	–	–	–	–	E	–
APQ	A	–	–	–	–	–
APQ-L_{247}	A	–	–	–	–	L
ASQ	A	–	–	S	–	–
Roe *Capreolus capreolus*						
TPQ	–	–	–	–	–	–
Sika *Cervus nippon*						
TPQ	–	–	–	–	–	–
Fallow *Dama dama*						
TPE-N_{138}	–	–	N	–	E	–
Muntjac *Muntiacus reevesii*						
SPQ	S	–	–	–	–	–
Chinese water deer *Hydropotes intermis*						
TPQ	–	–	–	–	–	–
TPQ-N_{100}	–	N	–	–	–	–

Muntjac and Chinese water deer

The *PRNP* ORF of all 41 muntjac deer analysed encoded serine at position 98, allowing their *PRNP* haplotype to be described as $S_{98}P_{168}Q_{226}$ (SPQ). In addition, synonymous polymorphisms were identified at nucleotide positions 15 (c/t), 126 (g/a) and 606 (c/t) at varying frequencies. The *PRNP* coding region of 27 Chinese water deer revealed two amino acid sequence polymorphisms; a serine to asparagine substitution at codon 100 (g/a, nucleotide position 299) and a 24 bp deletion of the octapeptide repeat (ccccatggaggtggctggggtcag) which corresponds to the in-frame loss of the amino acid sequence PHGGGWGQ. In most species, a glycine-rich peptide sequence (octapeptide repeat) is present in five consecutive copies in the N terminal region of wild type PrP^C, whilst Chinese water deer had a four octapeptide repeat haplotype. From the genotypes it was inferred that the deletion was linked to 100S (100S-Δ_{71-78}). The haplotype frequencies were 0.21 for 100S, 0.25 for 100SΔ_{71-78}, and 0.54 for 100 N. Seven animals were heterozygous for 100SΔ_{71-78}, whilst three were homozygous $S_{100}\Delta_{71-78}$/$S_{100}\Delta_{71-78}$. The most common genotype in this sampling was N_{100}/N_{100} (0.35).

Red deer genotype and haplotype frequencies and distributions

Red deer showed the greatest variation in *PRNP* sequence amongst the six deer species surveyed, with four haplotypes identified in the British population. Regional variation was evident within Great Britain in the distribution and frequency of the four *PRNP* haplotypes and resulting genotypes (Tables 2 and 3). For the TPQ and TPE haplotypes, frequencies in different regions of Scotland and England ranged from 0.11 to 0.62 and 0.31 to 0.88 respectively. The difference was most striking in Southern England, which had a very high frequency (0.88) of the TPE haplotype, whereas in Northern England, the TPQ haplotype was predominant (0.67). The APQ and ASQ haplotypes were found almost exclusively in Scottish red deer, with only one heterozygous TPE/APQ individual identified in Northern England. The frequencies of APQ and ASQ haplotypes showed regional variation within Scotland, with the highest frequencies seen in the Central Highlands, Argyll and Southern Scotland (Table 3). When compared using the Chi squared test, the Central Highlands, Lowlands and Argyll were significantly different in haplotype frequency when compared to the Northern Highlands and Hebrides ($P < 0.05$), as were the overall haplotype frequencies of England and Scotland ($P < 0.05$).

The haplotype frequencies in GB were also compared by Chi squared test with those in red deer from Norway and the Czech Republic, as well as previously published surveys of Scotland, Italy and Spain [46, 47] (Table 4). The number of samples was too low to assess the extent of regional variation within Norway and the Czech

Table 2 Genotype frequencies of *PRNP* polymorphisms in British red deer populations

98	168	226	England			Scotland					
			Total	S	N	Total	SS	A	CH	NH	H
TT	PP	EE	58 (64)	78 (61)	9 (3)	29 (107)	31 (7)	28 (13)	13 (19)	41 (47)	48 (21)
TT	PP	QE	26 (28)	19 (15)	41 (13)	28 (102)	22 (5)	15 (7)	28 (39)	34 (39)	27 (12)
TT	PP	QQ	15 (17)	3 (2)	47 (15)	10 (36)	9 (2)	8 (4)	11 (15)	10 (11)	9 (4)
TA	PP	QE	1 (1)	–	3 (1)	14 (52)	26 (6)	19 (9)	16 (23)	9 (10)	9 (4)
TA	PP	QQ	–	–	–	8 (31)	4 (1)	13 (6)	11 (16)	5 (6)	5 (2)
AA	PP	QQ	–	–	–	3 (13)	–	11 (5)	4 (6)	1 (1)	2 (1)
TA	PS	QE	–	–	–	4 (14)	4 (1)	4 (2)	8 (11)	–	–
TA	PS	QQ	–	–	–	2 (8)	4 (1)	2 (1)	4 (6)	–	–
AA	PS	QQ	–	–	–	2 (7)	–	–	5 (7)	–	–
AA	SS	QQ	–	–	–	–	–	–	–	–	–
			100 (110)	100 (78)	100 (32)	100 (370)	100 (23)	100 (47)	100 (142)	100 (114)	100 (44)

S: Southern England, N: Northern England, SS: Southern Scotland, A: Argyll, CH: Central Highlands, NH: Northern Highlands, H: Hebrides.
Percentage frequencies are given with animal numbers in brackets.

Table 3 Haplotype frequencies of *PRNP* polymorphisms in British red deer populations

Haplotype	Southern England	Northern England	Southern Scotland	Argyll	Central Highlands	Northern Highlands	Hebrides	GB average
TPE	0.88	0.31	0.57	0.47	0.39	0.63	0.66	0.56
TPQ	0.12	0.67	0.24	0.23	0.32	0.29	0.25	0.29
APQ	0	0.02	0.15	0.27	0.20	0.08	0.09	0.12
ASQ	0	0	0.04	0.03	0.09	0	0	0.03
Total	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Republic. In Norway, where only the TPQ and TPE haplotypes were identified, haplotype frequencies were very similar to those found in Southern England, i.e. 0.89 for TPE and 0.11 for TPQ. In the Czech Republic, frequencies of the TPQ and TPE haplotypes were approximately equal (0.35 and 0.44, respectively), while the APQ and APQ-L_{247} haplotypes were present at frequencies of 0.16 and 0.05, respectively. The APQ-L_{147} haplotype was only seen in the Czech red deer belonging to the Western lineage ($n=24$) [43]. The haplotype frequencies presented in Table 4 were compared across countries, and whilst most differences between countries were statistically significant ($P<0.05$), those in Spain and the Czech Republic were not.

Discussion

This study represents the largest survey to date of *PRNP* genetic variation in the wild (free-ranging) deer population of any European country and is the first to include free-ranging muntjac and Chinese water deer. The analysis included over 1003 animals from six different species of deer present in Britain, as well as red deer and sika deer from mainland Europe. Ten haplotypes were identified, five of which were present in red deer. The majority of polymorphisms identified in the ORF (exon 3) of the *PRNP* gene have been reported previously [32, 46–49], apart from the I247L polymorphism in red deer from the Czech Republic, and the octapeptide repeat deletion in Chinese water deer. Deletion of the third glycine-rich octapeptide repeat is not an uncommon variation, having previously been described in chamois [47], lions [50], and some primates [51]. This is the first time a functional octapeptide deletion variant has been described in deer.

Red deer showed the greatest variation in *PRNP* sequence, with four haplotypes identified in Great Britain, designated TPQ, TPE, APQ and ASQ. The TPQ, TPE and APQ haplotypes have been previously reported in surveys of Italian, Scottish (as haplotypes 1, 8 and 10) [46] and Spanish red deer [47]. The ASQ haplotype was not identified in Italy and Spain, and only reported previously in a single Scottish red deer [46]. Non-synonymous polymorphisms previously identified in Italian red deer at codons 59 (G59S) and 208 (M208I) in single individuals [46, 52], as well as low frequency synonymous

Table 4 Comparison of haplotype frequencies across European red deer populations

	TPE	TPQ	APQ	ASQ	Total animals	References
England	0.71	0.28	0[a]	0	110	
Scotland	0.52	0.29	0.16	0.04	370	
Scotland	0.69	0.27	0.05	0	132	[46]
Norway	0.89	0.11	0	0	50	
Czech	0.35	0.44	0.21[b]	0	46	
Spain	0.36	0.43	0.21	0	209	[47]
Italy	0.28	0.62	0.10	0	191	[46]

[a] A single animal was identified with the APQ genotype.

[b] The L_{247} polymorphism was present on an APQ background at a frequency of 0.05.

polymorphisms at codons 15, 21, 59, 78 and 79 [46] were not present in our surveyed populations.

Taking our results together with previously published surveys, the total number of European red deer analysed for *PRNP* sequence variation stands at 1108 individuals. In every region or country surveyed, TPQ and TPE haplotypes were present at the highest frequencies (Tables 3 and 4). In comparison to other European red deer populations, there was a high frequency of the TPE haplotype in South England (predominantly the Exmoor National Park) and in Norway, which is possibly due to historical population declines of red deer with refugial populations in these areas [53]. The APQ haplotype was absent from both Southern England and Norway but was found at similar frequencies in Scottish (0.16), Czech (0.16), Spanish (0.21) and Italian (0.10) red deer [46, 47]. In our study, it was evident that the frequency of the APQ haplotype showed marked variation between different Scottish regions (Table 3). This may explain why our frequency of the APQ haplotype was higher (0.16) than the previous Scottish survey (0.05); in the previous study, samples were collected from the Isle of Rum and an unspecified location on mainland Scotland, which may not have coincided with regions where we identified the highest APQ haplotype frequencies. The ASQ and APQ-L_{247} haplotypes were present at frequencies of 0.04 and 0.05 in Scottish and Czech red deer populations respectively and may be unique to these populations. Our results emphasize that there are large regional variations in *PRNP* genotype frequencies in British red deer populations, and similar variation in genotype frequencies between the red deer populations surveyed in European countries to date. Regional variations in the genetics of Scottish red deer populations have been previously reported, in part due to natural barriers [36] and in part due to introductions of non-native red deer into Scotland from England and further afield [54].

In contrast to red deer, our roe deer samples showed no variation in their *PRNP* sequence. Whilst the number of analysed animals across studies stands now at 541, only one synonymous change at codon 24 has previously been reported in two individuals [48]. Considering that roe deer are now the most widespread species in the UK, it is possible that this lack of diversity may be due to a population bottleneck following the near-extinction of British roe deer prior to the 1800 s [37]. However, a similar lack of *PRNP* diversity was also evident in European roe deer populations from Italy [46], Scandinavia [48] and Spain [47]. Similarly, we found little evidence of sequence variation in sika, fallow and muntjac deer, with the result that each of these species is predicted to express a single specific variant of the PrP protein. However, with a relatively small number of animals analysed (103, 92 and 41 respectively), less frequent polymorphisms may still be found in larger surveys. The *PRNP* sequence we identified in British fallow deer (described here as TPE-N_{138}), was identical to previously published sequences from Scandinavia [48] and Spain [47], as well as fallow deer previously used in experimental challenge experiments [34, 55]. Our data support the conclusion that the N_{138} change is fixed in the fallow deer sequence and not polymorphic. The three haplotypes identified in 27 wild Chinese water deer is more than might be expected considering that the UK population was established relatively recently from small numbers of escapees from captive collections.

Comparison of the *PRNP* genotypes in European deer with those in North American cervid species with European data can be used to help predict their potential susceptibility to CWD, although confirmation of these predictions under natural or experimental challenge is essential. The TPQ haplotype, which we identified at high frequencies in British red (0.29), Roe (1.0), Sika (1.0) and Chinese Water deer (0.21), is identical to *PRNP* sequences found in North American cervids that are highly susceptible to CWD. Our analysis, and published data to date for European deer species, has not

identified any *PRNP* polymorphisms that are associated with reduced CWD susceptibility in North American cervids, e.g. M132L, G96S [25]. This suggests that a relatively high proportion of free-ranging/wild deer in Britain and Europe may be susceptible to CWD, or at least to the predominant strains of CWD in North America.

The association of non-synonymous *PRNP* polymorphisms found only in British/European deer with susceptibility to CWD has yet to be determined. Amino acid substitutions at codon 98 are found in red (T98A) and muntjac (T98S) deer, and nearby codons 95 and 96 play a role in CWD susceptibility in white-tailed deer [24]. However, muntjac deer with identical *PRNP* sequences can be experimentally infected with North American CWD isolates [31] and recently camels, which encode alanine at the equivalent position, were reported to develop natural prion disease [56].

The substitution of serine for proline at codon 168 in red deer is of particular interest, as this position is located in the β2-α2 loop of the prion protein, an area critical for protein structure and stability [57]. The murine prion protein with a serine substitution at the equivalent residue showed no difference to wildtype in efficiency of conversion to PrP^{Sc} in an in vitro cell-free conversion assay [57]. However, in sheep the substitution of leucine for proline at codon 168 was associated with very prolonged incubation periods for experimental BSE infection [58], and a reduction of cell-free conversion efficiencies [59]. The ASQ haplotype seen in red deer has not been tested in vitro or by prion challenge, so any potential association with prion disease is still unresolved.

The novel *PRNP* haplotype TPQ-L_{247} identified in Czech red deer may be less likely to influence susceptibility as it represents a conservative amino acid substitution within the C-terminal signal peptide which is cleaved from the mature prion protein [60], however it is possible that it may have unknown effects that influence pathogenesis.

In North America codon 226 encodes either E (e.g. wapiti) or Q (e.g. mule deer and white-tailed deer), whereas in European red deer both variants are present and heterozygous animals are common. Codon 226 is in the third α-helix of PrP^{C}, a part of the protein involved in pathogenic processes during prion disease. Nearby polymorphisms include S225F, which is associated with reduced CWD susceptibility in mule deer [20], and Q222K, which is strongly associated with prion disease resistance in goats [61, 62]. Studies in transgenic mice expressing cervid PrP with Q226 or E226 suggest that variation at 226 may contribute to differences in disease progression and susceptibility to certain prion strains [63, 64]. Following experimental oral transmission of CWD to four deer of EE_{226}, QQ_{226} and EQ_{226} genotypes, all animals became infected [65]. The small sample size of this study does not allow a definite conclusion regarding the association of codon 226 with CWD susceptibility, or the effect of heterozygosity at this position.

The TPQ-N_{100} haplotype identified in Chinese water deer has not been reported in any other cervid but N_{100} is common to other mammalian prion proteins including bank vole, a species highly susceptible to CWD challenges [66]. The fact that fallow deer failed to become infected with CWD when exposed to infected mule deer for up to 7 years suggests that the substitution of asparagine at codon 138 may be associated with relative resistance to infection, but it is possible that other species-specific genetic factors also play a role [34].

In summary, this study has provided an analysis of *PRNP* sequence variation in the six major deer species found in the wild in Great Britain and adds to our knowledge of *PRNP* variation in European deer species in general. Based on a comparison of the sequences with those found in North American cervids affected by CWD, it appears likely that a large proportion of the British deer population would be susceptible to CWD. The effect of specific *PRNP* haplotypes only found in European species, such as ASQ in Scottish red deer, on CWD susceptibility are still to be determined.

Abbreviations

CWD: chronic wasting disease; *PRNP*: prion protein gene; SNP: single nucleotide polymorphism; EU: European Union; GB: Great Britain; ORF: open reading frame.

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Authors' contributions

Study design and manuscript writing: ALR, JMP, WG, FH; sample collection and DNA preparation: ALR, HW, MEG, HT, KB, SLS, SP-E, JK-P; sample analysis: ALR, HW, MEG, HT, WG. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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References

1. Williams ES, Young S (1980) Chronic wasting disease of captive mule deer: a spongiform encephalopathy. J Wildl Dis 16:89–98
2. Williams ES, Miller MW (2002) Chronic wasting disease in deer and elk in North America. Rev Sci Tech 21:305–316
3. 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
4. USGS (2018) Distribution of chronic wasting disease in North America Sept 2018. https://www.usgs.gov/media/images/distribution-chronic-wasting-disease-north-america-sept-2018. Accessed 22 Oct 2018
5. Lee Y-H, Sohn H-J, Kim M-J, Kim H-J, Lee W-Y, Yun E-I, Tark D-S, Cho I-S, Balachandran A (2013) Strain characterization of the Korean CWD cases in 2001 and 2004. J Vet Med Sci 75:95–98
6. EFSA (2010) Scientific opinion on the results of the EU survey for Chronic Wasting Disease (CWD) in cervids. EFSA J 8:1861
7. Benestad SL, Mitchell G, Simmons M, Ytrehus B, Vikoren T (2016) First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer. Vet Res 47:88
8. 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
9. Våge J, Hopp P, Vikoren T, Madslien K, Tarpai A, Moldal T, Benestad S (2018) The surveillance programme for Chronic Wasting Disease (CWD) in free ranging and captive cervids in Norway 2017 https://www.vetinst.no/overvaking/chronic-wasting-disease-vilt-cwd/_/attachment/. Accessed 20 Mar 2019
10. Evira (2018) Moose Found Dead in Forest with Chronic Wasting Disease https://mmm.fi/en/article/-/asset_publisher/evira-metsaan-kuolleella-hirvella-naivetystauti. Accessed 9 July 2019
11. SVA (2019) Wasting Disease (CWD) discovered on moose in Norrbotten County. https://www.sva.se/om-sva/pressrum/nyheter-fran-sva/avmagringssjuka-cwd-upptackt-pa-alg-i-norrbottens-lan. Accessed 1 Apr 2019 (in Swedish)
12. Ortiz-Pelaez A, Thompson CE, Dawson M, Hunter N (2014) The impact of the National Scrapie Plan on the PRNP genotype distribution of the British national flock, 2002–2012. Vet Rec 174:530
13. Dawson M, Del Rio Vilas V (2008) Control of classical scrapie in Great Britain. Practice 30:330–333
14. O'Rourke KI, Besser TE, Miller MW, Cline TF, Spraker TR, Jenny AL, Wild MA, Zebarth GL, Williams ES (1999) PrP genotypes of captive and free-ranging Rocky Mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease. J Gen Virol 80:2765–2769
15. Perucchini M, Griffin K, Miller MW, Goldmann W (2008) PrP genotypes of free-ranging wapiti (*Cervus elaphus nelsoni*) with chronic wasting disease. J Gen Virol 89:1324–1328
16. Hamir AN, Gidlewski T, Spraker TR, Miller JM, Creekmore L, Crocheck M, Cline T, O'Rourke KI (2006) Preliminary observations of genetic susceptibility of elk (*Cervus elaphus nelsoni*) to chronic wasting disease by experimental oral inoculation. J Vet Diagn Invest 18:110–114
17. O'Rourke KI, Spraker TR, Zhuang D, Greenlee JJ, Gidlewski TE, Hamir AN (2007) Elk with a long incubation prion disease phenotype have a unique PrP d profile. NeuroReport 18:1935–1938
18. Green KM, Browning SR, Seward TS, Jewell JE, Ross DL, Green MA, Williams ES, Hoover EA, Telling GC (2008) The elk PRNP codon 132 polymorphism controls cervid and scrapie prion propagation. J Gen Virol 89:598–608
19. Brayton KA, O'Rourke KI, Lyda AK, Miller MW, Knowles DP (2003) A processed pseudogene contributes to apparent mule deer prion gene heterogeneity. Gene 326:167–173
20. Jewell JE, Conner MM, Wolfe LL, Miller MW, Williams ES (2005) Low frequency of PrP genotype 225SF among free-ranging mule deer (*Odocoileus hemionus*) with chronic wasting disease. J Gen Virol 86:2127–2134
21. Fox KA, Jewell JE, Williams ES, Miller MW (2006) Patterns of PrPCWD accumulation during the course of chronic wasting disease infection in orally inoculated mule deer (*Odocoileus hemionus*). J Gen Virol 87:3451–3461
22. Wolfe LL, Fox KA, Miller MW (2014) "Atypical" chronic wasting disease in *PRNP* genotype 225FF mule deer. J Wildl Dis 50:660–665
23. 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
24. Johnson C, Johnson J, Vanderloo JP, Keane D, Aiken JM, McKenzie D (2006) Prion protein polymorphisms in white-tailed deer influence susceptibility to chronic wasting disease. J Gen Virol 87:2109–2114
25. Robinson SJ, Samuel MD, O'Rourke KI, Johnson CJ (2012) The role of genetics in chronic wasting disease of North American cervids. Prion 6:153–162
26. Johnson CJ, Herbst A, Duque-Velasquez C, Vanderloo JP, Bochsler P, Chappell R, McKenzie D (2011) Prion protein polymorphisms affect chronic wasting disease progression. PLoS One 6:e17450
27. Keane DP, Barr DJ, Bochsler PN, Hall SM, Gidlewski T, O'Rourke KI, Spraker TR, Samuel MD (2008) Chronic wasting disease in a Wisconsin white-tailed deer farm. J Vet Diagn Invest 20:698–703
28. Otero A, Duque Velásquez C, Johnson C, Herbst A, Bolea R, Badiola JJ, Aiken J, McKenzie D (2019) Prion protein polymorphisms associated with reduced CWD susceptibility limit peripheral PrPCWD deposition in orally infected white-tailed deer. BMC Vet Res 15:50
29. Martin S, Jeffrey M, González L, Sisó S, Reid HW, Steele P, Dagleish MP, Stack MJ, Chaplin MJ, Balachandran A, Gonzalez L, Siso S, Reid HW, Steele P, Dagleish MP, Stack MJ, Chaplin MJ, Balachandran A (2009) Immunohistochemical and biochemical characteristics of BSE and CWD in experimentally infected European red deer (*Cervus elaphus elaphus*). BMC Vet Res 5:26
30. 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
31. Nalls AV, McNulty E, Powers J, Seelig DM, Hoover C, Haley NJ, Hayes-Klug J, Anderson K, Stewart P, Goldmann W, Hoover EA, Mathiason CK (2013) Mother to offspring transmission of chronic wasting disease in reeves' muntjac deer. PLoS One 8:e71844
32. Nalls AV, McNulty E, Hoover CE, Pulscher LA, Hoover EA, Mathiason CK (2017) Infectious prions in the pregnancy microenvironment of chronic wasting disease-infected reeves' muntjac deer. J Virol 91:e00501–e00517
33. Hamir AN, Greenlee JJ, Nicholson EM, Kunkle RA, Richt JA, Miller JM, Hall M (2011) Experimental transmission of chronic wasting disease (CWD) from elk and white-tailed deer to fallow deer by intracerebral route: final report. Can J Vet Res 75:152–156
34. Rhyan JC, Miller MW, Spraker TR, McCollum M, Nol P, Wolfe LL, Davis TR, Creekmore L, O'Rourke KI (2011) Failure of fallow deer (*Dama dama*) to develop chronic wasting disease when exposed to a contaminated environment and infected mule deer (*Odocoileus hemionus*). J Wildl Dis 47:739–744
35. Pérez-Espona S, Pemberton JM, Putman R (2009) Red and sika deer in the British Isles, current management issues and management policy. Mamm Biol 74:247–262
36. Pérez-Espona S, Pérez-Barbería FJ, Mcleod JE, Jiggins CD, Gordon IJ, Pemberton JM (2008) Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). Mol Ecol 17:981–996
37. Baker KH, Hoelzel A (2013) Evolution of population genetic structure of the British roe deer by natural and anthropogenic processes (*Capreolus capreolus*). Ecol Evol 3:89–102

38. Smith SL, Senn HV, Pérez-Espona S, Wyman MT, Heap E, Pemberton JM (2018) Introgression of exotic Cervus (*nippon* and *canadensis*) into red deer (*Cervus elaphus*) populations in Scotland and the English Lake District. Ecol Evol 8:2122–2134
39. Amos B, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. In: Genetic ecology of whales and dolphins. Report of the International Whaling Commission. pp 99–103
40. Senn HV, Pemberton JM (2009) Variable extent of hybridization between invasive sika (*Cervus nippon*) and native red deer (*C. elaphus*) in a small geographical area. Mol Ecol 18:862–876
41. Esin A, Bergendahl LT, Savolainen V, Marsh JA, Warnecke T (2018) The genetic basis and evolution of red blood cell sickling in deer. Nat Ecol Evol 2:367–376
42. Krojerova-Prokesova J, Barančeková M, Voloshina I, Myslenkov A, Lamka J, Koubek P (2013) Dybowski's sika deer (*Cervus nippon hortulorum*): genetic divergence between natural primorian and introduced Czech populations. J Hered 104:312–326
43. Krojerova-Prokesova J, Barančeková M, Koubek P (2015) Admixture of eastern and western European red deer lineages as a result of postglacial recolonization of the Czech Republic (Central Europe). J Hered 106:375–385
44. Tan BC, Blanco ARA, Fiona Houston E, Stewart P, Goldmann W, Gill AC, de Wolf C, Manson JC, McCutcheon S (2012) Significant differences in incubation times in sheep infected with bovine spongiform encephalopathy result from variation at codon 141 in the PRNP gene. J Gen Virol 93:2749–2756
45. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
46. Peletto S, Perucchini M, Acín C, Dalgleish MP, Reid HW, Rasero R, Sacchi P, Stewart P, Caramelli M, Ferroglio E, Bozzetta E, Meloni D, Orusa R, Robetto S, Gennero S, Goldmann W, Acutis PL (2009) Genetic variability of the prion protein gene (PRNP) in wild ruminants from Italy and Scotland. J Vet Sci 10:115–120
47. Pitarch JL, Raksa HC, Arnal MC, Revilla M, Martínez D, Fernández De Luco D, Badiola JJ, Goldmann W, Acín C (2018) Low sequence diversity of the prion protein gene (PRNP) in wild deer and goat species from Spain. Vet Res 49:33
48. 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
49. Jeong H-J, Lee J-B, Park S-Y, Song C-S, Kim B-S, Rho J-R, Yoo M-H, Jeong B-H, Kim Y-S, Choi I-S (2009) Single-nucleotide polymorphisms in prion protein gene of the Korean subspecies of Chinese water deer (*Hydropotes inermis argyropus*). Korean J Vet Res 49:59–62
50. Stewart P, Campbell L, Skogtvedt S, Griffin KA, Arnemo JM, Tryland M, Girling S, Miller MW, Tranulis MA, Goldmann W (2012) Genetic predictions of prion disease susceptibility in carnivore species based on variability of the prion gene coding region. PLoS One 7:e50623
51. Schätzl HM, Da Costa M, Taylor L, Cohen FE, Prusiner SB (1995) Prion protein gene variation among primates. J Mol Biol 245:362–374
52. Kaluz S, Kaluzova M, Flint APF (1997) Sequencing analysis of prion genes from red deer and camel. Gene 199:283–286
53. Haanes H, Røed KH, Flagstad Rosef O (2010) Genetic structure in an expanding cervid population after population reduction. Conserv Genet 11:11–20
54. Pérez-Espona S, Hall RJ, Pérez-Barbería FJ, Glass BC, Ward JF, Pemberton JM (2013) The impact of past introductions on an iconic and economically important species, the red deer of Scotland. J Hered 104:14–22
55. Hamir AN, Kunkle RA, Nicholson EM, Miller JM, Hall SM, Schoenenbruecher H, Brunelle BW, Richt JA (2008) Preliminary observations on the experimental transmission of chronic wasting disease (CWD) from elk and white-tailed deer to fallow deer. J Comp Pathol 138:121–130
56. Babelhadj B, Di Bari MA, Pirisinu L, Chiappini B, Gaouar SBS, Riccardi G, Marcon S, Agrimi U, Nonno R, Vaccari G (2018) Prion disease in dromedary camels, Algeria. Emerg Infect Dis 24:1029–1036
57. Kirby L, Agarwal S, Graham JF, Goldmann W, Gill AC (2010) Inverse correlation of thermal lability and conversion efficiency for five prion protein polymorphic variants. Biochemistry 49:1448–1459
58. Goldmann W, Houston F, Stewart P, Perucchini M, Foster J, Hunter N (2006) Ovine prion protein variant A136R154L168Q171 increases resistance to experimental challenge with bovine spongiform encephalopathy agent. J Gen Virol 87:3741–3745
59. Kirby L, Goldmann W, Houston F, Gill AC, Manson JC (2006) A novel, resistance-linked ovine PrP variant and its equivalent mouse variant modulate the in vitro cell-free conversion of rPrP to PrPres. J Gen Virol 87:3747–3751
60. Gill AC, Castle AR (2018) The cellular and pathologic prion protein. Handb Clin Neurol 153:21–44
61. Lacroux C, Perrin-Chauvineau C, Corbière F, Aron N, Aguilar-Calvo P, Torres JM, Costes P, Brémaud I, Lugan S, Schelcher F, Barillet F, Andréoletti O (2014) Genetic resistance to scrapie infection in experimentally challenged goats. J Virol 88:2406–2413
62. Aguilar-Calvo P, Fast C, Tauscher K, Espinosa J-C, Groschup MH, Nadeem M, Goldmann W, Langeveld J, Bossers A, Andreoletti O, Torres J-M (2015) Effect of Q_{211} and K_{222} *PRNP* polymorphic variants in the susceptibility of goats to oral infection with goat bovine spongiform encephalopathy. J Infect Dis 212:664–672
63. Angers RC, Seward TS, Napier D, Green M, Hoover E, Spraker T, O'Rourke K, Balachandran A, Telling GC (2009) Chronic wasting disease prions in elk antler velvet. Emerg Infect Dis 15:696–703
64. Angers RC, Kang H-E, Napier D, Browning S, Seward T, Mathiason C, Balachandran A, McKenzie D, Castilla J, Soto C, Jewell J, Graham C, Hoover EA, Telling GC (2010) Prion strain mutation determined by prion protein conformational compatibility and primary structure. Science 328:1154–1158
65. 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 (*Census elaphus elaphus*): early detection and late stage distribution of protease-resistant prion protein. Can Vet J 51:169–178
66. Di Bari MA, Nonno R, Castilla J, D'Agostino C, Pirisinu L, Riccardi G, Conte M, Richt J, Kunkle R, Langeveld J, Vaccari G, Agrimi U (2013) Chronic wasting disease in bank voles: characterisation of the shortest incubation time model for prion diseases. PLoS Pathog 9:e1003219

Publisher's Note

Article IV

Title

Chronic wasting disease in Norway – a survey of prion protein gene variation among cervids

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Summary

Susceptibility of cervids to Chronic Wasting Disease (CWD), a prion disease, can be modulated by variations in the prion protein gene (*PRNP*), encoding the cellular prion protein (PrP^{C}). In prion diseases, PrP^{C} is conformationally converted to pathogenic conformers (PrP^{Sc}), aggregates of which comprise infectious prions. CWD has recently been observed in its contagious form in Norwegian reindeer (*Rangifer tarandus*) and in novel, potentially sporadic forms, here called "atypical CWD", in moose (*Alces alces*) and red deer (*Cervus elaphus*). To estimate relative susceptibility of different Norwegian cervid species to CWD, their non-synonymous *PRNP* variants were analyzed. In reindeer, seven *PRNP* alleles were observed and in red deer and moose two alleles were present, whereas roe deer (*Capreolus capreolus*) *PRNP* was monomorphic. One "archetypal" *PRNP* allele associated with susceptibility was common to all four cervid species. The distribution of *PRNP* alleles differed between wild and semi-domesticated reindeer, with alleles associated with a high susceptibility occurring, on average, above 55% in wild reindeer and below 20% in semi-domesticated reindeer. This difference may reflect the diverse origins of the populations and/or selection processes during domestication and breeding. Overall, *PRNP* genetic data indicate considerable susceptibility to CWD among Norwegian cervids and suggest that *PRNP* homozygosity may be a risk factor for the atypical CWD observed in moose. The CWD isolates found in the Norwegian cervid species differ from those previously found in Canada and USA, indicating that CWD in Norway is unlikely to be a recent introduction from North America. Our study provides an overview of the *PRNP* genetics in populations exposed to these emerging strains that will provide a basis for understanding these strains' dynamics in relation to *PRNP* variability.

Keywords

Prion Proteins; Genetic Variation; Alleles; Wasting Disease, Chronic; Deer; Norway

Introduction

Chronic wasting disease (CWD, MONDO_0002680) is an incurable prion disease of cervids (Williams & Young, 1980). Like other prion diseases, CWD is caused by misfolding of the neuroprotective cellular prion protein (PrP^{C}) to a pathogenic conformer, (PrP^{Sc}), aggregates of which constitute transmissible prions (Bolton, McKinley, & Prusiner, 1982; Prusiner, 1998; Prusiner et al., 1990; Stahl & Prusiner, 1991). Since its discovery in the early 1960s, CWD has spread relentlessly in North America affecting various members of the family *Cervidae*; in addition, there have been reports of CWD in the Republic of Korea, Norway, Sweden, and Finland (Rivera, Brandt, Novakofski, & Mateus-Pinilla, 2019). The disease can be transmitted horizontally directly between animals or indirectly, via prion-contaminated environments (Gough & Maddison, 2010; Nicholas J. Haley et al., 2011). The prolonged survival of prions in the environment, along with the lack of treatment or prophylaxis, mean that control and eradication are very challenging (Uehlinger, Johnston, Bollinger, & Waldner, 2016). CWD management has proven difficult, especially once the disease is established. It can thus have a long-term negative impact on the population sustainability (DeVivo et al., 2017; Edmunds et al., 2016). Like classical scrapie in sheep and goats, which is also a contagious prion disease, susceptibility of cervids to CWD is influenced by variations in the host prion protein gene (*PRNP*) encoding PrP^{C} (Goldmann, 2008; S. J. Robinson, Samuel, O'Rourke, & Johnson, 2012). Amino acid substitutions at codons 225 and 226 in the cervid PrP^{C} primary structure have been shown to cause structural arrangements that could preclude PrP^{C} to PrP^{Sc} conversion (Bian et al., 2019), thus modulating CWD susceptibility.

Among commonly studied cervids, those with the higher CWD prevalence are mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and elk (wapiti; *Cervus elaphus nelsoni*) (Rivera et al., 2019). These species share a consensus primary structure of PrP^{C}, differing only at codon 226 with either glutamine (Q; mule and white-tailed deer) or glutamate (E; elk) (Bian et al., 2019). These two PrP^{C} amino acid sequences (*226Q*, *226E*) are also predominant in many other cervid species, including reindeer, moose, red deer, roe deer, and some species of Neotropical deer (Falcão et al., 2017; A. L. Robinson et al., 2019; S. J. Robinson, Samuel, O'Rourke, et al., 2012; Wik et al., 2012). Additional *PRNP* variants arise from non-synonymous substitutions on the backbone of *226Q* and *226E* (Martin,

Gallet, Rocha, & Petit, 2009) and these variants have been shown to vary geographically (Arifin et al., 2020; Miller & Walter, 2019). Possible factors driving these distribution patterns may include adaptive processes and demographic features (Miller & Walter, 2019). In cervid species with reported natural CWD infections, the number of variant codons ranges from one in elk, two in moose and mule deer, three in red deer, four in white-tailed deer, and six in reindeer/caribou (Güere et al., 2020; S. J. Robinson, Samuel, O'Rourke, et al., 2012; Wik et al., 2012). Substitutions in a single variant site can significantly influence CWD susceptibility. The substitutions Met132Leu in elk (S. J. Moore et al., 2018; White, Spraker, Reynolds, & O'Rourke, 2010) and Gly96Ser in white-tailed deer (Nicholas J. Haley et al., 2019) are, for example, associated with a lower risk and slower progression of CWD. In CWD-affected populations, *226Q* and *226E* are consistently overrepresented among cases, despite the coexistence of additional *PRNP* variants, such as those encoding substitutions (Güere et al., 2020; Nicholas J. Haley et al., 2019; Jewell, Conner, Wolfe, Miller, & Williams, 2005; White et al., 2010). This suggests that *226Q* and *226E* increase susceptibility to CWD.

Disease progression and manifestation are also influenced by the strain of prion (Bessen & Marsh, 1994; Bruce, 1993; Collinge, 2010; Collinge & Clarke, 2007). At least two CWD strains have been identified in North America (Duque Velásquez et al., 2015; J. Moore et al., 2020; Raymond et al., 2007). CWD isolates from Norwegian reindeer and moose are not only different from each other, but also differ from the North American strains (Nonno et al., 2020; Pirisinu et al., 2018; Wadsworth et al., 2021). Thus, the occurrence of CWD in Europe, identified in 2016, did not reflect a simple territorial expansion of North American CWD prions, but the emergence of new variants of the agent (prion strains). Moreover, the moose strain found in Fennoscandia might represent a sporadic form of CWD, affecting only old animals, with PrP^{Sc} in the brain but not in lymphoid tissues, and being potentially less contagious (Pirisinu et al., 2018; Ågren et al., 2021). This disease modality is reminiscent of that of Nor98/atypical scrapie (Benestad et al., 2003) and atypical forms of bovine spongiform encephalopathy in cattle (Biacabe, Laplanche, Ryder, & Baron, 2004; Casalone et al., 2004; Dudas & Czub, 2017). However, these forms in cattle are not considered contagious. In addition, a single case of CWD was diagnosed in a 16-year-old Norwegian red deer in 2017, again with unusual features such as the peculiar

distribution pattern of PrP^{Sc} (Vikøren et al., 2019). The recent observation of a further Norwegian CWD case in reindeer, outside the initial outbreak area (ProMED-mail, 2020), indicates that CWD can be present at very low levels, escaping rigorous surveillance, and posing a potential threat to cervid populations throughout Europe.

To predict the susceptibility of Norwegian cervid populations that are potentially exposed to these novel CWD strains, a survey of *PRNP* genetic variation was performed in the four endemic cervid species in Norway (reindeer, red deer, moose, and roe deer). These species are differently distributed and managed within Norway, with red deer, moose, and roe deer largely managed as wild animals (some red deer are farmed). These cervid species are almost continuously distributed across most of Norway, although red deer and roe deer are uncommon in some areas and are scarce in North Norway (Linnell et al., 2020). In contrast, reindeer are managed both as wild and semi-domesticated, each having different management units. Whereas wild reindeer are partially isolated in 24 different mountainous areas in South-central Norway (Kvie, Heggenes, Bårdsen, & Røed, 2019), semi-domestic reindeer are herded under several reindeer husbandry districts and concession areas in north (majority) and central Norway (Næss and Bårdsen (2015) and https://www.fylkesmannen.no/en/portal/Agriculture-and-food/Reindeer-husbandry/). Mapping *PRNP* variations among Norwegian reindeer, red deer, moose, and roe deer may identify subpopulations that are more susceptible to CWD. Here, we aimed to describe *PRNP* variability among Norwegian cervid species and evaluate whether the genetic structure of these populations might affect the distribution of *PRNP* variants. In addition, we wanted to explore factors that may explain differences in *PRNP* variation between subpopulations.

Materials and methods

Study population

The study included 653 animals (reindeer, red deer, moose, and roe deer) sampled in the locations illustrated in **Figure 1**. Reindeer originated from 12 subpopulations (**Figure 1A**, **Table 1**) encompassing seven wild reindeer areas (Kvie et al., 2019): Rondane (1:w), Snøhetta (2:w), Sølnkletten (3:w), Hardangervidda (4:w), Nordfjella (5:w), Setesdal-Austhei (6:w) and Forollhogna (7:w); and five reindeer husbandry locations Filefjell Reinlag (8:d), (9:d), Færen (10:d), Beahcegealli (11:d) and Nordkinnhalvøya/Vestertana (12:d). For the other species, the sampling sites corresponded to 40 municipalities for red deer (**Figure 1B**), 47 for moose (**Figure 1C**), and 30 for roe deer (**Figure 1D**). Further details on the sampling distribution can be found in **Supplementary table 1**.

Samples of most reindeer, together with all those from red deer, moose, and roe deer, were brain tissue collected through the CWD-surveillance program during the hunting seasons 2016 to 2018 (including some animals killed in traffic) (Våge et al., 2020). These were examined for CWD at the Norwegian Veterinary Institute as described by Güere et al. (2020). To increase representation of wild reindeer, the dataset included *PRNP* sequencing data from 50 animals that had tested CWD PrP^{Sc} negative and that were randomly selected from a previous case-control study in Nordfjella (Güere et al., 2020). Similarly, additional DNA samples obtained in 2005-2006 from wild reindeer in Sølnkletten and Snøhetta (Knut H Røed et al., 2008) and in 2012-2013 from Hardangervidda and Setesdal-Austhei (Kvie et al., 2019; Kvie, Heggenes, & Røed, 2016) were also used. All samples were regarded as CWD PrP^{Sc} negative.

In addition, we analyzed CWD cases (PrP^{Sc} positive) that had been diagnosed up until March 2021 with unreported *PRNP* genotypes. These included a wild reindeer shot in September 2020 in Hardangervidda area (**Figure 1A**), and five moose with death date between 2018 and 2020 in the municipalities of Flesberg, Selbu, Sigdal, Steinkjer and Bamble (**Figure 1C**).

PRNP sequencing

For samples gathered from 2016 and onwards, including the CWD cases in reindeer and moose, genomic DNA was isolated using DNeasy® Blood & Tissue Kit (Qiagen, Oslo, Norway) according to

the manufacturer's instructions. DNA was available for samples from prior to 2016 and that had been similarly extracted (Kvie et al., 2019; Kvie et al., 2016); DNA from samples from 2005-2006 had been extracted according to a standard chloroform: phenol procedure (Knut H Røed et al., 2008). The DNA served as the template for amplification and sequencing of the open reading frame of *PRNP* with PCR primers Ce19_F and Ce778_ R (O'Rourke et al., 1999), following the *PRNP* sequencing methodology specified by Güere et al. (2020) to optimize their visualization range.

The sequencing data were aligned and edited using SeqScape v3.0 software (Applied Biosystems) and MEGA7 version 7.0.26 (Kumar, Stecher, & Tamura, 2016) respectively. The variant positions and alleles are described at the nucleotide- and protein level based on reference sequences: DQ154293.1 and AAZ81474.1 for reindeer, KT845862.1 and AMP43861.1 for red deer, MH230115.1 and AZB50215.1 for moose, and MK103016.1 and QAU19526.1 for roe deer. Data on variant positions in *PRNP* (including linkage disequilibrium analyses and *PRNP* allele descriptions as either DNA or protein variants) are found in **Supplementary: table 2** – **3**; **figure 1**.

Data and statistical analyses

The data and statistical analyses were processed using R version 4.0.3 (R Core Team, 2018). The *tidyverse* (Wickham et al., 2019) and *plyr* (Wickham, 2011) packages served as the basis for data handling and plotting.

The sequences were interpreted as unphased genotypes from which two alleles per individual were inferred. The aligned DNA sequences were handled using the *Biostrings* package to select the variant positions for each species. The nucleotide symbol in the variant position was transformed into the correspondent nucleotide pair (e.g., Y into thymine and cytosine), before the allele and genotype frequencies were determined using the *genetics* package. Those variant positions encoding a non-synonymous change were selected to infer the *PRNP* variants. In species with two or more non-synonymous variant sites (reindeer and red deer), such positions further underwent pairwise analysis of linkage disequilibrium (LD), available in the *genetics* package, considering the minimum number of

alleles as in Güere et al. (2020). After inferring the *PRNP* alleles in each species, the allele and genotype frequencies were calculated using the *genetics* package.

We tested phased genotypes for HWE (Hardy-Weinberg Equilibrium) using Fisher's exact test in the *genetics* package. For the HWE test of reindeer genotypes, we used the Markov chain Monte Carlo (MCMC) algorithm (1700 iterations, 100 batches of size 170) (Guo & Thompson, 1992) available in the *gap* package. Differences in *PRNP* variants between groups were analyzed using Fisher's exact test (two-sided), with Bonferroni correction for multiple comparisons if applied. The null-hypothesis was rejected at an α-level of 0.050. Under Bonferroni correction, the α-level was divided by the number of comparisons to determine significance.

For red deer and moose samples, we performed a spatial analysis of the *PRNP* genotypes, where the geographic coordinates (X= longitude, Y = latitude) of the centroid of the municipality of origin (under projection arguments: "+proj=longlat +ellps=WGS84") were used as continuous predictors. We applied a multinomial logistic regression modelling approach, using the *multinom* function in the *nnet* package (Venables and Ripley (2002); see also Fox (2002) for description of the analyses) to estimate the probabilities for the various *PRNP* genotypes occurring in the sampling area. The response variable represents the *PRNP* genotype as a categorical variable, choosing those genotypes similar to A/A in reindeer (i.e., *226Q*/*226Q* in red deer and *109K*/*109K* in moose) as the baseline in the corresponding analyses for each species. The rationale for choosing the baseline category was the higher CWD risk associated with this genotype. Additional categories in the response variable included *226E*/*226Q* and *226E*/*226E* in red deer and *109K*/*109Q* and *109Q*/*109Q* in moose.

Complementary plotting packages served to generate the sequence logos: *ggseqlogo*; and to construct the sampling and spatial interpolation maps: *geojsonio*, *rgdal*, *ggsn* and *maptools*.

Results

The study population consisted of 365 reindeer, 105 red deer, 137 moose, and 46 roe deer that had been sampled across Norway (**Figure 1**). As well as some variation at the *PRNP* nucleotide level, occurring as synonymous substitutions, there was variation at the nucleotide level affecting the *PRNP* amino acid sequences in reindeer, red deer, and moose. Roe deer *PRNP*, however, was monomorphic. The *PRNP* variants were analyzed and reported as alleles with unique amino acid sequences (**Figure 2**).

Seven *PRNP* alleles were observed in reindeer, among which five were termed: *A*, *B* (p.Ser225Tyr), *C* (p.Trp84_Gly91del), *D* (p.Asn176Asp), and *E* (p.Val2Met;Gly129Ser;Val169Met), as reported by Güere et al. (2020), and two novel alleles named *F* (p.Lys207Met) and *G* (p.Arg211Gln). The allele *G* was observed in only one animal (heterozygote D/G) and this animal was excluded from further analysis (*n*=364). Two *PRNP* alleles were found in red deer, *226Q* and *226E* (p.Ala136=;Gln226Glu), and in moose, *109K* and *109Q* (p. Lys109Gln). The four cervid species encoded a *PRNP* allele with an identical amino acid sequence, represented by the allele *A* in reindeer, *226Q* in red deer, *109K* in moose, and the fixed allele in roe deer (**Figure 2**), despite synonymous single nucleotide variants (SNVs) between them. The proportion of this *PRNP* allele differed between species, ranging from fixation in roe deer to 0.11 in red deer (**Figure 3**). Among the CWD cases recorded during 2018-2021 (March), the *PRNP* genotypes were *A*/*D* in the only reindeer, *109K*/*109K* and *109Q*/*109Q* in two and three moose respectively. These genotypes are plotted together with those found in the previous cases reported by Güere et al. (2020) and Pirisinu et al. (2018) in **Figure 4**.

Novel sequences have been submitted to the DDBJ/EMBL/GenBank databases under accession numbers MW557843 (*Rangifer tarandus tarandus* with 620A>T, predicted *A*/*F* genotype), MW557844 (*Rangifer tarandus tarandus* with 620A>T; 674 C>A, predicted *B*/*F* genotype) and MW557845 (*Rangifer tarandus tarandus* with 526A>G; 632G>A, predicted *D*/*G* genotype).

In reindeer, the six alleles identified in more than one animal (*A*-*F*, **Figure 2**) paired into 15 genotypes. The most abundant genotypes were *A*/*A* (0.198), *A*/*B* (0.184), and *B*/*B* (0.179) while other genotypes were detected at proportions <0.10. No animals homozygous for the alleles *C* or *F* were detected. *PRNP*

genotypes' frequencies departed from HWE (P<.001) in the overall population but attained HWE when sorted into the 12 sampling sites.

Comparing the *PRNP* allele distribution among the 12 reindeer subpopulations (**Table 1**) revealed a clear pattern of low or no significant differentiation between most wild subpopulations. The exception were Nordfjella (5:w) and Forollhogna (7:w), which respectively differed from either one or three other wild subpopulations (wild vs. wild, **Figure 5A**). Similarly, low or no genetic differentiation characterized the sample sets of five semi-domestic herds, except for Filefjell (8:d), which differed from the Beahcegealli herd (11:d) (semi-domestic vs semi-domestic, **Figure 5A**). On the contrary, the pairwise comparisons between wild and semi-domestic subpopulations showed significant differences from one another. When pooled as wild and semi-domestic, the *PRNP* allele distribution differences were highly significant (P<.001; **Figure 5B**). The allele *A* was the most prevalent in all wild subpopulations, except Forollhogna (7:w) where *B* was the dominating allele. Allele *B* dominated in semi-domestic herds with the exception of Filefjell (8:d), which had more of the allele *A* (**Table 1**). In addition, the alleles *D* and *E* appear at higher frequencies among semi-domestic reindeer (**Figure 5B**).

For both red deer and moose the two *PRNP* alleles combined into three genotypes that were in HWE (respectively P>.347 and .15, Fisher's exact test). *PRNP* variant *226E* was clearly the most common allele in the overall red deer population (0.89), while the *109K* was the dominating allele in moose (0.774; **Figure 3**). For both species, the predicted probabilities of observing the *PRNP* genotypes based on the multinomial model gave evidence of spatial trends (**Supplementary: table 4** – **5**; **figure 2**). Whereas, the common *PRNP* allele among Norwegian cervids in red deer (*226Q*) appears to be mostly distributed in the western range (i.e. between Storfjorden and Sognefjorden; **Figure 1B**), this allele for moose (*109K*) was mostly distributed in the northernmost and southernmost areas (i.e. north of Ofotfjorden and south of Trondheimsfjorden; **Figure 1C**). The estimated distribution in red deer and moose of either *PRNP* genotypes are given in **Supplementary data**.

Discussion

Among Norwegian cervids, reindeer have the highest number of non-synonymous variant positions in *PRNP*, with seven *PRNP* alleles, whereas red deer and moose both have two alleles, and roe deer was found to be monomorphic. Despite species differences in *PRNP* genetic variability and geographical distribution, all species had one *PRNP* allele encoding a consensus amino acid sequence. The spatial *PRNP* variation revealed a distinct difference between wild and semi-domestic reindeer, and red deer and moose were characterized by spatial trends, with increased frequency of the consensus *PRNP* allele in certain regions of these species' distribution.

The difference in *PRNP* variation between Norwegian wild and semi-domestic reindeer is congruent with studies of genetic structure and variation based on neutral genetic markers (Kvie et al., 2019; Knut H. Røed, Bjørklund, & Olsen, 2018; Knut H Røed et al., 2008). For instance, the wild Forollhogna reindeer (7:w), which is assumed to be domestic in origin (Nyaas, 2016), has *PRNP* variation similar to semi-domestic reindeer (**Figure 5A**). In contrast, reindeer of the semi-domestic Filefjell herd (8:d) show certain features of *PRNP* variation similar to those in wild reindeer. This may be the result of occasional contact (Strand et al., 2011) and subsequent introgression from the neighboring wild Nordfjella subpopulation (5:w; **Figure 1A**). The wild subpopulations 1:w-3:w are parts of the wild Rondane-Dovre population and are considered to have a wild ancestry primarily, with little introgression from semi-domestic reindeer (Kvie et al., 2019; Knut H. Røed et al., 2014). The almost total absence of alleles *D* and *E* from these subpopulations might suggest that these alleles are mostly associated with semi-domestic reindeer. However, this interpretation should be considered with caution, given that our study design did not considered the possible effects of distinctive ancestry in *PRNP* variability.

The higher frequencies of *226Q* in red deer located between fjords in western Norway, and that of *109K* in moose from areas north of Ofotfjorden and south of Trondheimsfjorden, are coincident with areas with a distinctive genetic signature when using neutral genetic markers (Haanes, Røed, Flagstad, & Rosef, 2010; Haanes, Røed, Solberg, Herfindal, & Sæther, 2011). Overall, the *PRNP* variation aligns well with the general genetic structure for the Norwegian cervid species, similar to reports from other

studies of *PRNP* variation in *Cervidae* (Arifin et al., 2020; Cheng, Musiani, Cavedon, & Gilch, 2017; Lang & Blanchong, 2012; Miller & Walter, 2019).

However, different adaptive processes associated with diverging selection affecting *PRNP* variation in Norwegian cervids should also be considered. *PRNP* variation may influence learning abilities, and long-term and visuospatial memory (Houlihan et al., 2009; Papassotiropoulos et al., 2005), consistent with proposed physiological roles for PrP^{C} in animal behavior and stress adaptation (Nico et al., 2005). Furthermore, it is possible that certain *PRNP* variants are functionally associated with traits that are desirable in semi-domestic reindeer, such as reduced fright responses (Reimers, Røed, & Colman, 2012), and thus have been selected for during domestication. On the other hand, in other cervids exposed to CWD there are indications of increased selection for less-susceptible *PRNP* alleles (Monello et al., 2017; S. J. Robinson, Samuel, Johnson, Adams, & McKenzie, 2012). The alleles *B* and *E*, common in semi-domestic reindeer, have been suggested to be associated with reduced CWD susceptibility (Güere et al., 2020; N. J. Haley et al., 2017; Mitchell et al., 2012). The substitution Ser225Tyr in allele *B* of reindeer resembles the protective Ser225Phe in mule deer (Jewell et al., 2005). Phenylalanine and tyrosine are structurally similar, with a characteristic aromatic ring and may have similar stabilizing effects on the long-range interaction between the C-terminal portion of α-helix-3 and the β2- α2 loop in PrP^{C} (Angers et al., 2014) likely delaying its PrP^{Sc} conversion (N. J. Haley et al., 2017). The higher frequencies of alleles *B* and *E* could reflect an adaptative response to an unknown CWD outbreak among semi-domestic reindeer or their ancestors. However, this controversial hypothesis needs stronger evidence regarding reduced susceptibility of these alleles to CWD. Of relevance to this theory is Met129Val in human *PRNP* that is associated with a reduced risk to certain prion diseases e.g. variant Creutzfeldt-Jakob (vCJD) and Kuru, and shows geographic stratification globally, which indicates that various factors might affect *PRNP* variability (Soldevila et al., 2006). Further investigations, for instance in behavioral physiology, may assist in clarifying the mechanisms underlying the large differences in *PRNP* allele distribution between wild and semi-domesticated reindeer.

Analysis of the Norwegian CWD prion isolates from wild reindeer and moose have shown these to differ from each other (Pirisinu et al., 2018) and from those found in North America (Nonno et al.,

2020). The disease observed in European moose may therefore represent a novel and sporadic form of CWD. Altogether, the eight CWD cases in Norwegian moose reported until March 2021 were homozygous for either allele *109K* (*n*=6) or *109Q* (*n*=2) (**Figure 4**), and the single case in red deer was homozygous for allele *226E* (Pirisinu et al., 2018; Vikøren et al., 2019). Although the numbers are small, these potentially sporadic cases are all *PRNP* homozygotes and this resembles the trend observed in sporadic Creutzfeldt-Jakob (sCJD) in humans where homozygosity at codon 129, i.e. either to methionine or valine, is considered a predisposing factor (Palmer, Dryden, Hughes, & Collinge, 1991). A proposed mechanism to explain this effect considers that a homologous PrP^{Sc} interaction may facilitate the process of amyloid formation (Hizume et al., 2009). It is not clear, however, whether such a process is relevant regarding the potentially sporadic CWD cases observed in Fennoscandia. Intriguingly, significant strain variability has been observed between moose CWD cases that have the same *PRNP* genotype (Nonno et al., 2020). This calls for further investigation of these emergent CWD strains and their transmission properties. Thus, CWD has emerged in Europe and is apparently evolving dynamically, with several novel strains and disease phenotypes, demonstrating the unpredictable nature of prion diseases. Our study provides information on *PRNP* variability in different species of cervids in Norway, giving an overview of local subpopulations with various levels of susceptibility to CWD conferred by their *PRNP* genotype. Finally, our data provides a basis against which future changes in *PRNP* variability in Norwegian cervids can be tracked and will assist in our understanding of the relationship between *PRNP* variation and the selection dynamics of CWD.

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Conflict of Interest

The authors declare no conflict of interest.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required. The genomic DNA used through this study was either obtained from brain tissues collected through the CWD-surveillance program at NVI or sourced from archived material at NMBU from previous studies (Kvie et al., 2019; Kvie et al., 2016; Knut H Røed et al., 2008).

Table 1. Alleles' frequencies of PRNP coding sequence in 12 subpopulations of Norwegian reindeer (n=364). The allele nomenclature corresponds to **Figure 2**.

Subpopulation	**Code**†	***n***‡	**Alleles proportion**§					
			A	B	C	D	E	F
Rondane	1:w	16	0.562	0.281	-	0.094	-	0.062
Snøhetta	2:w	22	0.773	0.182	0.045	-	-	-
Sølnkletten	3:w	29	0.638	0.224	0.017	-	-	0.121
Hardangervidda	4:w	47	0.691	0.149	0.021	0.085	0.053	-
Nordfjella	5:w	60	0.408	0.325	0.067	0.108	0.092	-
Setesdal-Austhei	6:w	8	0.375	0.375	-	0.062	0.188	-
Forollhogna	7:w	29	0.31	0.483	-	0.052	0.155	-
Filefjell Reinlag	8:d	29	0.397	0.362	0.017	0.086	0.138	-
Riast/Hylling	9:d	55	0.136	0.591	-	0.118	0.155	-
Færen	10:d	15	0.067	0.7	-	0.133	0.1	-
Beahcegealli	11:d	30	0.067	0.317	-	0.267	0.35	-
Nordkinnhalvøya/ Vestertana	12:d	24	0.083	0.396	-	0.229	0.292	-

† Wild (:w) or semi-domestic (:d)

‡ Number of animals

§ Relative frequency of *PRNP* alleles for each subpopulation

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Figure 3. Relative frequency of *PRNP* alleles (2*n*) in the Norwegian reindeer (*Rangifer tarandus*; *n*=364), red deer (*Cervus elaphus*; *n*=105), moose (*Alces alces*; *n*=137) and roe deer (*Capreolus capreolus*; *n*=46). The allele nomenclature corresponds to **Figure 2**. In yellow, the common *PRNP* allele shared among the four species.

Figure 4. Relative frequency of *PRNP* genotypes among CWD cases identified in Norwegian reindeer (*Rangifer tarandus*; *n*=20) and moose (*Alces alces*; *n*=8). The allele nomenclature corresponds to **Figure 2**. Based on the copy number of the common *PRNP* allele, the *PRNP* genotypes are colored yellow (two copies), dark yellow (one copy) and grey (no copies).

Figure 5. (A) *P*-values from pairwise comparisons of *PRNP* alleles' frequencies among 12 subpopulations of Norwegian reindeer. The significance levels are based on Fisher's exact test followed by Bonferroni correction colored dark gray when significant (*P*-value ≤0.05/66). The subpopulations nomenclature corresponds to **Table 1**. **(B)** Relative frequency of *PRNP* alleles (2n) in the Norwegian wild and semi-domestic reindeer. The allele nomenclature corresponds to **Figure 2**. In yellow, the common *PRNP* allele shared among reindeer, red deer, moose, and roe deer.

References

Angers, R., Christiansen, J., Nalls, A. V., Kang, H.-E., Hunter, N., Hoover, E., . . . Telling, G. C. (2014). Structural effects of PrP polymorphisms on intra- and interspecies prion transmission. *Proceedings of the National Academy of Sciences, 111*(30), 11169-11174. doi:https://doi.org/10.1073/pnas.1404739111

Arifin, M. I., Staskevicius, A., Shim, S. Y., Huang, Y. H., Fenton, H., McLoughlin, P. D., . . . Gilch, S. (2020). Large-scale prion protein genotyping in Canadian caribou populations and potential impact on chronic wasting disease susceptibility. *Molecular Ecology, 00*, 1-11. doi:https://doi.org/10.1111/mec.15602

Benestad, S. L., Sarradin, P., Thu, B., Schönheit, J., Tranulis, M. A., & Bratberg, B. (2003). Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. *Veterinary Record, 153*(7), 202-208. doi:https://doi.org/10.1136/vr.153.7.202

Bessen, R. A., & Marsh, R. F. (1994). Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *Journal of Virology, 68*(12), 7859-7868. Retrieved from https://jvi.asm.org/content/jvi/68/12/7859.full.pdf

Biacabe, A.-G., Laplanche, J.-L., Ryder, S., & Baron, T. (2004). Distinct molecular phenotypes in bovine prion diseases. *EMBO reports, 5*(1), 110-115. doi:https://doi.org/10.1038/sj.embor.7400054

Bian, J., Christiansen, J. R., Moreno, J. A., Kane, S. J., Khaychuk, V., Gallegos, J., . . . Telling, G. C. (2019). Primary structural differences at residue 226 of deer and elk PrP dictate selection of distinct CWD prion strains in gene-targeted mice. *Proceedings of the National Academy of Sciences, 116*(25), 12478-12487. doi:https://doi.org/10.1073/pnas.1903947116

Bolton, D., McKinley, M., & Prusiner, S. (1982). Identification of a protein that purifies with the scrapie prion. *Science, 218*(4579), 1309-1311. doi:https://doi.org/10.1126/science.6815801

Bruce, M. E. (1993). Scrapie strain variation and mutation. *British Medical Bulletin, 49*(4), 822-838. doi:https://doi.org/10.1093/oxfordjournals.bmb.a072649

Casalone, C., Zanusso, G., Acutis, P., Ferrari, S., Capucci, L., Tagliavini, F., . . . Caramelli, M. (2004). Identification of a second bovine amyloidotic spongiform encephalopathy: Molecular similarities with sporadic Creutzfeldt-Jakob disease. *Proceedings of the National Academy of Sciences, 101*(9), 3065-3070. doi:https://doi.org/10.1073/pnas.0305777101

Cheng, Y. C., Musiani, M., Cavedon, M., & Gilch, S. (2017). High prevalence of prion protein genotype associated with resistance to chronic wasting disease in one Alberta woodland caribou population. *Prion, 11*(2), 136-142. doi:https://doi.org/10.1080/19336896.2017.1300741

Collinge, J. (2010). Prion Strain Mutation and Selection. *Science, 328*(5982), 1111-1112. doi:https://doi.org/10.1126/science.1190815

Collinge, J., & Clarke, A. R. (2007). A General Model of Prion Strains and Their Pathogenicity. *Science, 318*(5852), 930-936. doi:https://doi.org/10.1126/science.1138718

DeVivo, M. T., Edmunds, D. R., Kauffman, M. J., Schumaker, B. A., Binfet, J., Kreeger, T. J., . . . Cornish, T. E. (2017). Endemic chronic wasting disease causes mule deer population decline in Wyoming. *PLOS ONE, 12*(10), e0186512. doi:https://doi.org/10.1371/journal.pone.0186512

Dudas, S., & Czub, S. (2017). Atypical BSE: Current Knowledge and Knowledge Gaps. *Food Safety, 5*(1), 10-13. doi:https://doi.org/10.14252/foodsafetyfscj.2016028

Duque Velásquez, C., Kim, C., Herbst, A., Daude, N., Garza, M. C., Wille, H., . . . McKenzie, D. (2015). Deer Prion Proteins Modulate the Emergence and Adaptation of Chronic Wasting Disease Strains. *Journal of Virology, 89*(24), 12362-12373. doi:https://doi.org/10.1128/JVI.02010-15

Edmunds, D. R., Kauffman, M. J., Schumaker, B. A., Lindzey, F. G., Cook, W. E., Kreeger, T. J., . . . Cornish, T. E. (2016). Chronic Wasting Disease Drives Population Decline of White-Tailed Deer. *PLOS ONE, 11*(8), e0161127. doi:https://doi.org/10.1371/journal.pone.0161127

Falcão, C. B. R., Lima, I. L. d. M. N. F., Duarte, J. M. B., de Oliveira, J. R. M., Torres, R. A., Wanderley, A. M., . . . Garcia, J. E. (2017). Are Brazilian cervids at risk of prion diseases? *Prion, 11*(1), 65-70. doi:https://doi.org/10.1080/19336896.2016.1274000

Fox, J. (2002). *R and S-Plus Companion to Applied Regression* (1 ed.). Thousand Oaks, CA: Sage Publications, Inc.

Goldmann, W. (2008). PrP genetics in ruminant transmissible spongiform encephalopathies. *Vet. Res., 39*(4), 30. doi:https://doi.org/10.1051/vetres:2008010

Gough, K. C., & Maddison, B. C. (2010). Prion transmission. *Prion, 4*(4), 275-282. doi:https://doi.org/10.4161/pri.4.4.13678

Guo, S. W., & Thompson, E. A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics, 48*(2), 361-372. doi:https://doi.org/10.2307/2532296

Güere, M. E., Våge, J., Tharaldsen, H., Benestad, S. L., Vikøren, T., Madslien, K., . . . Tranulis, M. A. (2020). Chronic wasting disease associated with prion protein gene (*PRNP*) variation in Norwegian wild reindeer (*Rangifer tarandus*). *Prion, 14*(1), 1-10. doi:https://doi.org/10.1080/19336896.2019.1702446

Haanes, H., Røed, K. H., Flagstad, Ø., & Rosef, O. (2010). Genetic structure in an expanding cervid population after population reduction. *Conservation Genetics, 11*(1), 11-20. doi:https://doi.org/10.1007/s10592-008-9781-0

Haanes, H., Røed, K. H., Solberg, E. J., Herfindal, I., & Sæther, B.-E. (2011). Genetic discontinuities in a continuously distributed and highly mobile ungulate, the Norwegian moose. *Conservation Genetics, 12*(5), 1131. doi:https://doi.org/10.1007/s10592-011-0214-0

Haley, N. J., Mathiason, C. K., Carver, S., Zabel, M., Telling, G. C., & Hoover, E. A. (2011). Detection of Chronic Wasting Disease Prions in Salivary, Urinary, and Intestinal Tissues of Deer: Potential Mechanisms of Prion Shedding and Transmission. *Journal of Virology, 85*(13), 6309-6318. doi:https://doi.org/10.1128/jvi.00425-11

Haley, N. J., Merrett, K., Buros Stein, A., Simpson, D., Carlson, A., Mitchell, G., . . . Thomsen, B. V. (2019). Estimating relative CWD susceptibility and disease progression in farmed white-tailed deer with rare PRNP alleles. *PLOS ONE, 14*(12), e0224342. doi:https://doi.org/10.1371/journal.pone.0224342

Haley, N. J., Rielinger, R., Davenport, K. A., O'Rourke, K., Mitchell, G., & Richt, J. A. (2017). Estimating chronic wasting disease susceptibility in cervids using real-time quaking-induced conversion. *J Gen Virol, 98*(11), 2882-2892. doi:https://doi.org/10.1099/jgv.0.000952

Hizume, M., Kobayashi, A., Teruya, K., Ohashi, H., Ironside, J. W., Mohri, S., & Kitamoto, T. (2009). Human Prion Protein (PrP) 219K Is Converted to PrP^{Sc} but Shows Heterozygous Inhibition in Variant Creutzfeldt-Jakob Disease Infection *. *Journal of Biological Chemistry, 284*(6), 3603-3609. doi:https://doi.org/10.1074/jbc.M809254200

Houlihan, L. M., Harris, S. E., Luciano, M., Gow, A. J., Starr, J. M., Visscher, P. M., & Deary, I. J. (2009). Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936. *Genes Brain Behav, 8*(2), 238-247. doi:https://doi.org/10.1111/j.1601-183X.2008.00470.x

Jewell, J. E., Conner, M. M., Wolfe, L. L., Miller, M. W., & Williams, E. S. (2005). Low frequency of PrP genotype 225SF among free-ranging mule deer (*Odocoileus hemionus*) with chronic wasting disease. *Journal of General Virology, 86*(8), 2127-2134. doi:https://doi.org/10.1099/vir.0.81077-0

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution, 33*(7), 1870-1874. doi:https://doi.org/10.1093/molbev/msw054

Kvie, K. S., Heggenes, J., Bårdsen, B.-J., & Røed, K. H. (2019). Recent large-scale landscape changes, genetic drift and reintroductions characterize the genetic structure of Norwegian wild reindeer. *Conservation Genetics, 20*, 1405–1419. doi:https://doi.org/10.1007/s10592-019-01225-w

Kvie, K. S., Heggenes, J., & Røed, K. H. (2016). Merging and comparing three mitochondrial markers for phylogenetic studies of Eurasian reindeer (*Rangifer tarandus*). *Ecology and Evolution, 6*(13), 4347-4358. doi:https://doi.org/10.1002/ece3.2199

Lang, K. R., & Blanchong, J. A. (2012). Population genetic structure of white-tailed deer: Understanding risk of chronic wasting disease spread. *The Journal of Wildlife Management, 76*(4), 832-840. doi:https://doi.org/10.1002/jwmg.292

Linnell, J. D. C., Cretois, B., Nilsen, E. B., Rolandsen, C. M., Solberg, E. J., Veiberg, V., . . . Kaltenborn, B. (2020). The challenges and opportunities of coexisting with wild ungulates in the human-dominated landscapes of Europe's Anthropocene. *Biological Conservation, 244*, 108500. doi:https://doi.org/10.1016/j.biocon.2020.108500

Martin, R., Gallet, P.-F., Rocha, D., & Petit, D. (2009). Polymorphism of the Prion Protein in Mammals: A Phylogenetic Approach. *Recent Patents on DNA & Gene Sequences, 3*(1), 63-71. doi:https://doi.org/10.2174/187221509787236156

Miller, W. L., & Walter, W. D. (2019). Spatial heterogeneity of prion gene polymorphisms in an area recently infected by chronic wasting disease. *Prion, 13*(1), 65-76. doi:https://doi.org/10.1080/19336896.2019.1583042

Mitchell, G. B., Sigurdson, C. J., O'Rourke, K. I., Algire, J., Harrington, N. P., Walther, I., . . . Balachandran, A. (2012). Experimental Oral Transmission of Chronic Wasting Disease to Reindeer (*Rangifer tarandus tarandus*). *PLOS ONE, 7*(6), e39055. doi:https://doi.org/10.1371/journal.pone.0039055

Monello, R. J., Galloway, N. L., Powers, J. G., Madsen-Bouterse, S. A., Edwards, W. H., Wood, M. E., . . . Wild, M. A. (2017). Pathogen-mediated selection in free-ranging elk populations infected by chronic wasting disease. *Proceedings of the National Academy of Sciences, 114*(46), 12208-12212. doi:https://doi.org/10.1073/pnas.1707807114

Moore, J., Tatum, T., Hwang, S., Vrentas, C., West Greenlee, M. H., Kong, Q., . . . Greenlee, J. (2020). Novel Strain of the Chronic Wasting Disease Agent Isolated From Experimentally Inoculated Elk With LL132 Prion Protein. *Scientific Reports, 10*(1), 3148. doi:https://doi.org/10.1038/s41598-020-59819-1

Moore, S. J., Vrentas, C. E., Hwang, S., West Greenlee, M. H., Nicholson, E. M., & Greenlee, J. J. (2018). Pathologic and biochemical characterization of PrP^{Sc} from elk with *PRNP* polymorphisms at codon 132 after experimental infection with the chronic wasting disease agent. *BMC Veterinary Research, 14*(1), 80. doi:https://doi.org/10.1186/s12917-018-1400-9

Nico, P. B. C., de-Paris, F., Vinadé, E. R., Amaral, O. B., Rockenbach, I., Soares, B. L., . . . Bianchin, M. M. (2005). Altered behavioural response to acute stress in mice lacking cellular prion protein. *Behavioural Brain Research, 162*(2), 173-181. doi:https://doi.org/10.1016/j.bbr.2005.02.003

Nonno, R., Di Bari, M. A., Pirisinu, L., D'Agostino, C., Vanni, I., Chiappini, B., . . . Agrimi, U. (2020). Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America. *Proceedings of the National Academy of Sciences, 117*(49), 31417-31426. doi:https://doi.org/10.1073/pnas.2013237117

Nyaas, A. (2016). Villreinområder i Fastlands-Norge. In *Villreinen - fjellviddas nomade* (pp. 448). Arendal: Friluftsforlaget.

Næss, M. W., & Bårdsen, B.-J. (2015). Market Economy vs. Risk Management: How Do Nomadic Pastoralists Respond to Increasing Meat Prices? *Human Ecology, 43*(3), 425-438. doi:https://doi.org/10.1007/s10745-015-9758-9

O'Rourke, K. I., Besser, T. E., Miller, M. W., Cline, T. F., Spraker, T. R., Jenny, A. L., . . . Williams, E. S. (1999). PrP genotypes of captive and free-ranging Rocky Mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease. *Journal of General Virology, 80*(10), 2765-2679. doi:https://doi.org/10.1099/0022-1317-80-10-2765

Palmer, M. S., Dryden, A. J., Hughes, J. T., & Collinge, J. (1991). Homozygous prion protein genotype predisposes to sporadic Creutzfeldt–Jakob disease. *Nature, 352*(6333), 340-342. doi:https://doi.org/10.1038/352340a0

Papassotiropoulos, A., Wollmer, M. A., Aguzzi, A., Hock, C., Nitsch, R. M., & de Quervain, D. J.-F. (2005). The prion gene is associated with human long-term memory. *Human Molecular Genetics, 14*(15), 2241-2246. doi:https://doi.org/10.1093/hmg/ddi228

Pirisinu, L., Tran, L., Chiappini, B., Vanni, I., DiBari, M. A., Vaccari, G., . . . Benestad, S. L. (2018). Novel Type of Chronic Wasting Disease Detected in Moose (*Alces alces*), Norway. *Emerging Infectious Disease journal, 24*(12), 2210-2218. doi:https://doi.org/10.3201/eid2412.180702

ProMED-mail. (2020). Chronic wasting disease - Europe: (Norway) reindeer. *ProMED-mail 2020, 12 Sep: 20200912.7773103*. Retrieved from http://www.promedmail.org

Prusiner, S. B. (1998). Prions. *Proceedings of the National Academy of Sciences, 95*(23), 13363-13383. doi:https://doi.org/10.1073/pnas.95.23.13363

Prusiner, S. B., Scott, M., Foster, D., Pan, K.-M., Groth, D., Mirenda, C., . . . DeArmond, S. J. (1990). Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell, 63*(4), 673-686. doi:https://doi.org/10.1016/0092-8674(90)90134-Z

R Core Team. (2018). R: A Language and Environment for Statistical Computing (Version 3.5.2). Vienna, Austria. Retrieved from https://www.R-project.org/

Raymond, G. J., Raymond, L. D., Meade-White, K. D., Hughson, A. G., Favara, C., Gardner, D., . . . Caughey, B. (2007). Transmission and Adaptation of Chronic Wasting Disease to Hamsters and Transgenic Mice: Evidence for Strains. *Journal of Virology, 81*(8), 4305-4314. doi:https://doi.org/10.1128/jvi.02474-06

Reimers, E., Røed, K. H., & Colman, J. E. (2012). Persistence of vigilance and flight response behaviour in wild reindeer with varying domestic ancestry. *Journal of Evolutionary Biology, 25*(8), 1543-1554. doi:https://doi.org/10.1111/j.1420-9101.2012.02538.x

Rivera, N. A., Brandt, A. L., Novakofski, J. E., & Mateus-Pinilla, N. E. (2019). Chronic Wasting Disease In Cervids: Prevalence, Impact And Management Strategies. *Veterinary medicine (Auckland, N.Z.), 10*, 123-139. doi:https://doi.org/10.2147/VMRR.S197404

Robinson, A. L., Williamson, H., Güere, M. E., Tharaldsen, H., Baker, K., Smith, S. L., . . . Houston, F. (2019). Variation in the prion protein gene (*PRNP*) sequence of wild deer in Great Britain and mainland Europe. *Veterinary Research, 50*(1), 59. doi:https://doi.org/10.1186/s13567-019-0675-6

Robinson, S. J., Samuel, M. D., Johnson, C. J., Adams, M., & McKenzie, D. I. (2012). Emerging prion disease drives host selection in a wildlife population. *Ecological Applications, 22*(3), 1050-1059. doi:https://doi.org/10.1890/11-0907.1

Robinson, S. J., Samuel, M. D., O'Rourke, K. I., & Johnson, C. J. (2012). The role of genetics in chronic wasting disease of North American cervids. *Prion, 6*(2), 153-162. doi:https://doi.org/10.4161/pri.19640

Røed, K. H., Bjørklund, I., & Olsen, B. J. (2018). From wild to domestic reindeer – Genetic evidence of a non-native origin of reindeer pastoralism in northern Fennoscandia. *Journal of Archaeological Science: Reports, 19*, 279-286. doi:https://doi.org/10.1016/j.jasrep.2018.02.048

Røed, K. H., Bjørnstad, G., Flagstad, Ø., Haanes, H., Hufthammer, A. K., Jordhøy, P., & Rosvold, J. (2014). Ancient DNA reveals prehistoric habitat fragmentation and recent domestic introgression into native wild reindeer. *Conservation Genetics, 15*(5), 1137-1149. doi:https://doi.org/10.1007/s10592-014-0606-z

Røed, K. H., Flagstad, Ø., Nieminen, M., Holand, Ø., Dwyer, M. J., Røv, N., & Vilà, C. (2008). Genetic analyses reveal independent domestication origins of Eurasian reindeer. *Proceedings of the Royal Society B: Biological Sciences, 275*(1645), 1849-1855. doi:https://doi.org/10.1098/rspb.2008.0332

Soldevila, M., Andrés, A. M., Ramírez-Soriano, A., Marquès-Bonet, T., Calafell, F., Navarro, A., & Bertranpetit, J. (2006). The prion protein gene in humans revisited: Lessons from a worldwide resequencing study. *Genome Research, 16*(2), 231-239. doi:https://doi.org/10.1101/gr.4345506

Speed, J. D. M., Austrheim, G., Kolstad, A. L., & Solberg, E. J. (2019). Long-term changes in northern large-herbivore communities reveal differential rewilding rates in space and time. *PLOS ONE, 14*(5), e0217166. doi:https://doi.org/10.1371/journal.pone.0217166

Stahl, N., & Prusiner, S. B. (1991). Prions and prion proteins1. *The FASEB Journal, 5*(13), 2799-2807. doi:https://doi.org/10.1096/fasebj.5.13.1916104

Strand, O., Jordhøy, P., Mossing, A., Knudsen, P. A., Nesse, L., Skjerdal, H., . . . Gundersen, V. (2011). *Villreinen i Nordfjella. Status og leveområde.* (1504-3312). Retrieved from Trondheim, Norway: http://www.nina.no/archive/nina/PppBasePdf/rapport/2011/634.pdf

Uehlinger, F. D., Johnston, A. C., Bollinger, T. K., & Waldner, C. L. (2016). Systematic review of management strategies to control chronic wasting disease in wild deer populations in North

America. *BMC Veterinary Research, 12*(1), 173. doi:https://doi.org/10.1186/s12917-016-0804-7

Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S* (Fourth Edition ed.). New York, NY: Springer, New York, NY.

Vikøren, T., Våge, J., Madslien, K. I., Røed, K. H., Rolandsen, C. M., Tran, L., . . . Benestad, S. L. (2019). First Detection of Chronic Wasting Disease in a Wild Red Deer (*Cervus elaphus*) in Europe. *Journal of Wildlife Diseases, 55*(4), 970-972. doi:https://doi.org/10.7589/2018-10-262

Våge, J., Hopp, P., Vikøren, T., Madslien, K., Tarpai, A., Moldal, T., & Benestad, S. L. (2020). *The surveillance programme for Chronic Wasting Disease (CWD) in free-ranging and captive cervids in Norway 2019* (1894-5678). Retrieved from Oslo, Norway: https://www.vetinst.no/en/surveillance-programmes/chronic-wasting-disease

Wadsworth, J. D. F., Joiner, S., Linehan, J. M., Jack, K., Al-Doujaily, H., Costa, H., . . . Collinge, J. (2021). Humanized Transgenic Mice Are Resistant to Chronic Wasting Disease Prions From Norwegian Reindeer and Moose. *The Journal of Infectious Diseases*, jiab033. doi:https://doi.org/10.1093/infdis/jiab033

White, S. N., Spraker, T. R., Reynolds, J. O., & O'Rourke, K. I. (2010). Association analysis of *PRNP* gene region with chronic wasting disease in Rocky Mountain elk. *BMC Res Notes, 3*(1), 314. doi:https://doi.org/10.1186/1756-0500-3-314

Wickham, H. (2011). The Split-Apply-Combine Strategy for Data Analysis. *Journal of Statistical Software, 40*(1), 29. doi:https://doi.org/10.18637/jss.v040.i01

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., . . . Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software, 4*(43), 1686. doi:https://doi.org/10.21105/joss.01686

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*(3), 256-260. doi:https://doi.org/10.4161/pri.19641

Williams, E. S., & Young, S. (1980). CHRONIC WASTING DISEASE OF CAPTIVE MULE DEER: A SPONGIFORM ENCEPHALOPATHY. *Journal of Wildlife Diseases, 16*(1), 89-98. doi:https://doi.org/10.7589/0090-3558-16.1.89

Ågren, E. O., Sörén, K., Gavier-Widén, D., Benestad, S. L., Tran, L., Wall, K., . . . Nöremark, M. (2021). First Detection of Chronic Wasting Disease in Moose (*Alces alces*) in Sweden. *Journal of Wildlife Diseases, 57*(2), 461-463. doi:https://doi.org/10.7589/JWD-D-20-00141

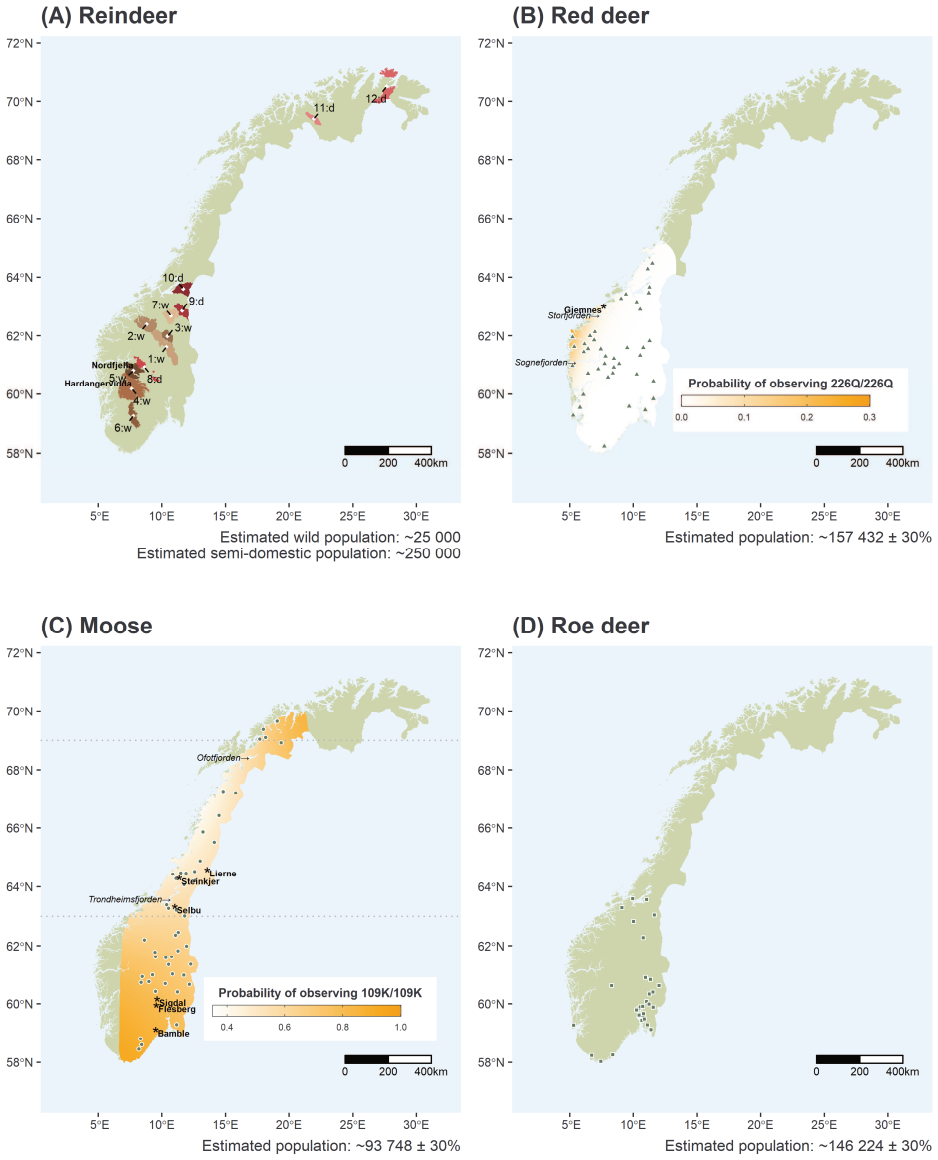


*Figure 1. Geographic distribution of sampling sites. For reindeer (**A**), these are represented as zones corresponding either to wild (brown palette; 1:w-7:w) or semi-domestic (red palette; 8:d-12:d) subpopulations, which nomenclature corresponds to **Table 1**. For red deer (**B**), moose (**C**) and roe deer (**D**), the sampling sites are represented as icons coincident with the midpoint of a sampled municipality. The horizontal lines indicate the latitudinal divisions of the Norwegian moose population previously described by Haanes et al. (2011). The allele nomenclature corresponds to **Figure 2**. The probability of observing 226Q/226Q in red deer (**B**) and 109K/109K in moose (**C**), which are similar to genotype A/A in reindeer -increased Chronic Wasting Disease (CWD) risk), are plotted as prediction maps based on multinomial logistic regression modelling. The color bars have been adjusted to better display the range of predicted probabilities for each genotype. Areas (reindeer) and municipalities (* in red deer and moose) with overall CWD cases up to 31.03.2021 are labeled. The population size estimates for red deer, moose, and roe deer correspond to 2015 and are based on analyses used in Speed, Austrheim, Kolstad, and Solberg (2019) (personal communication, Erling Solberg).*

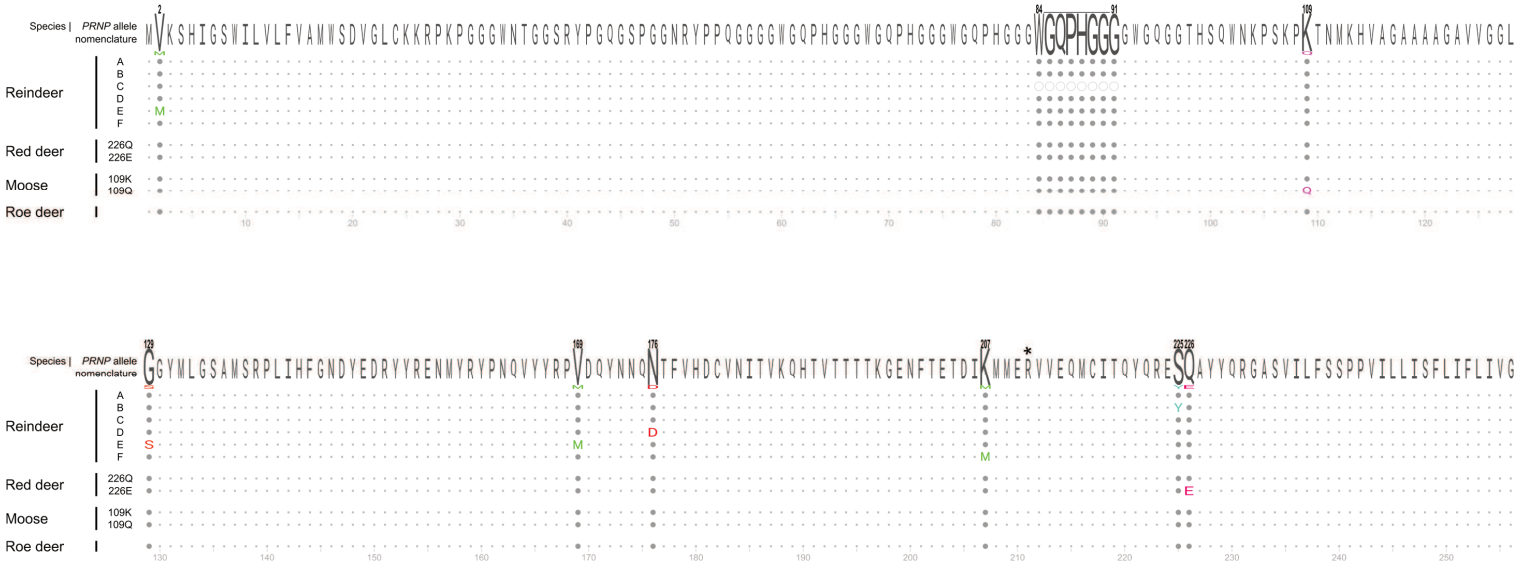


Figure 2. *Amino acid sequence alignment of the prion protein gene (PRNP) alleles in Norwegian cervids. Variant positions in reindeer (Rangifer tarandus), red deer (Cervus elaphus) and moose (Alces alces) but not in roe deer (Capreolus capreolus) are zoomed in and either numbered on top for amino acid substitutions. The octapeptide deletion is pointed out with a horizontal line. An additional variant position (* on top) was identified in one reindeer allele with Arg211Gln compared with the reference sequence. The 256 amino acids from the PRNP coding sequence are displayed using a one-letter amino acid code. Alleles identical to the reference sequence (AAZ81474.1 for reindeer, AMP43861.1 for red deer, AZB50215.1 for moose and QAU19526.1 for roe deer) head the list for each species. There is a consensus stop codon at position 257 across all species (not displayed).*

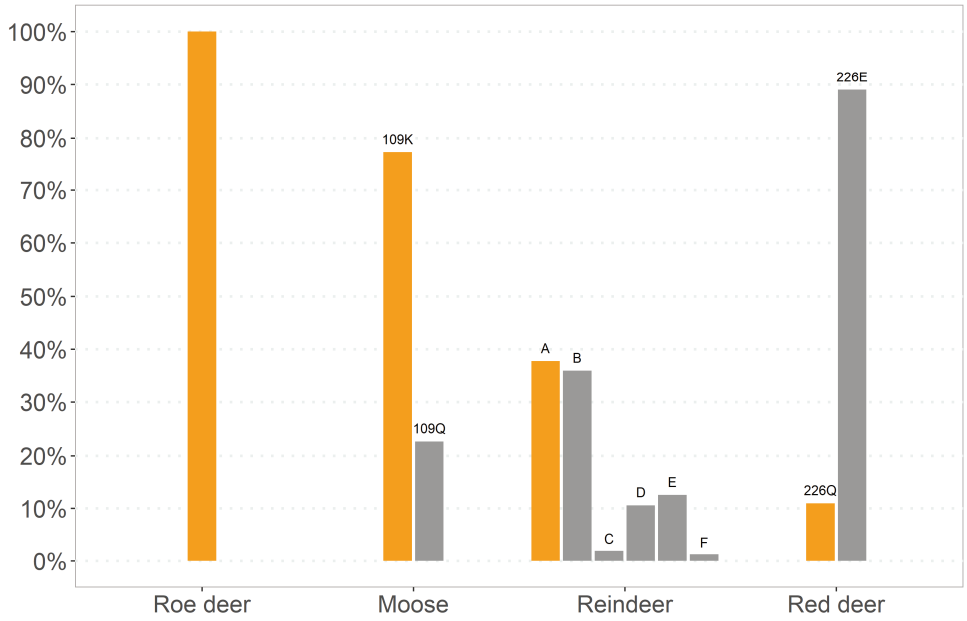


Figure 3. *Relative frequency of PRNP alleles (2n) in the Norwegian reindeer (Rangifer tarandus; n=364), red deer (Cervus elaphus; n=105), moose (Alces alces; n=137) and roe deer (Capreolus capreolus; n=46). The allele nomenclature corresponds to* ***Figure 2****. In yellow, the common PRNP allele shared among the four species.*

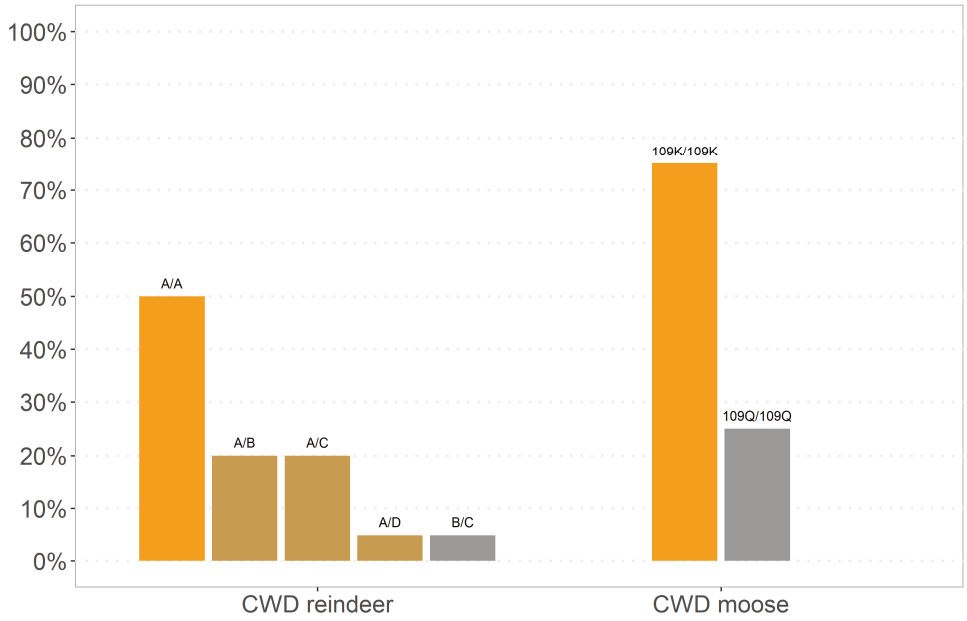


Figure 4. *Relative frequency of PRNP genotypes among CWD cases identified in Norwegian reindeer (Rangifer tarandus; n=20) and moose (Alces alces; n=8). The allele nomenclature corresponds to* ***Figure 2****. Based on the copy number of the common PRNP allele, the PRNP genotypes are colored yellow (two copies), dark yellow (one copy) and grey (no copies).*

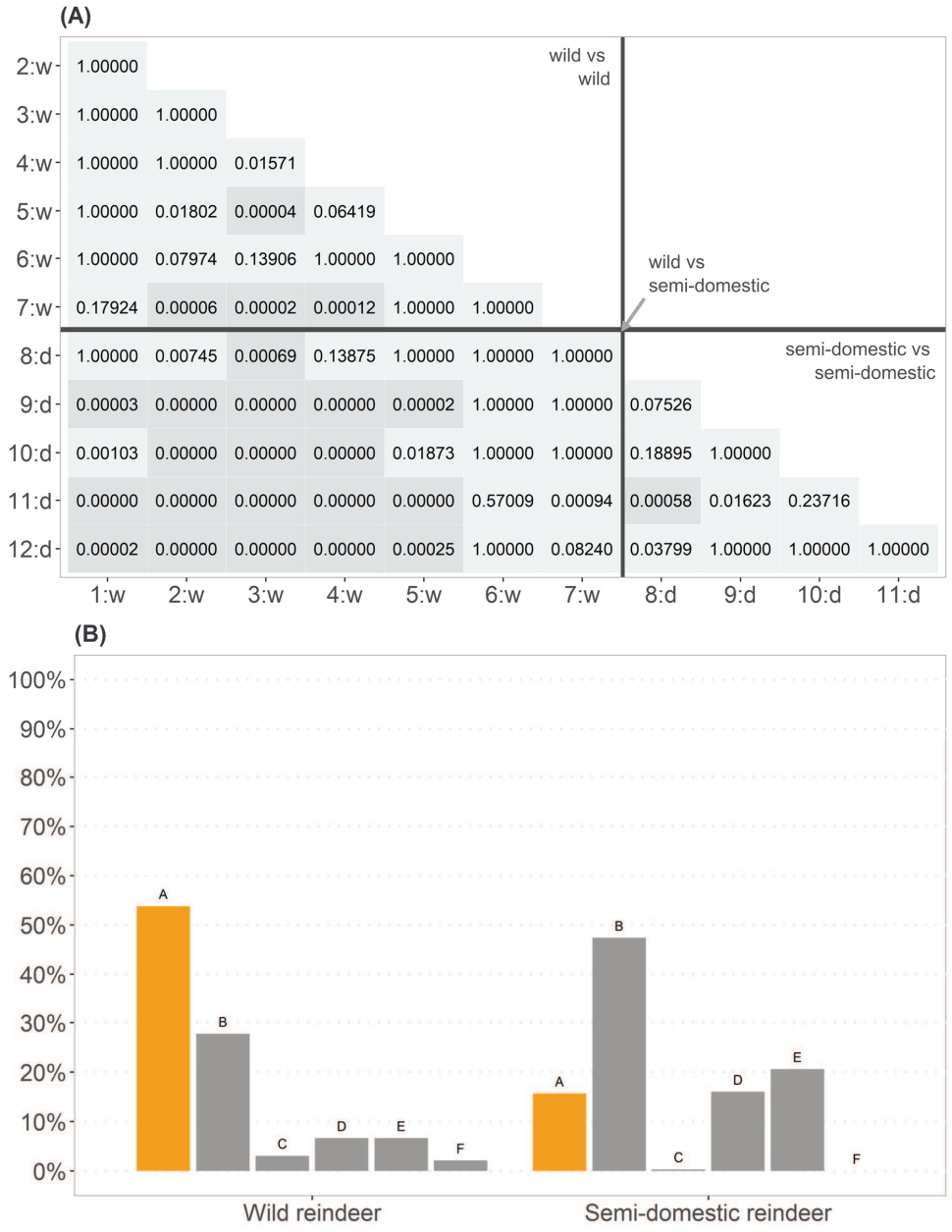


Figure 5. *(**A**) P-values from pairwise comparisons of PRNP alleles' frequencies among 12 subpopulations of Norwegian reindeer. The significance levels are based on Fisher's exact test followed by Bonferroni correction colored dark gray when significant (P-value ≤0.05/66). The subpopulations nomenclature corresponds to* ***Table 1****. (**B**) Relative frequency of PRNP alleles (2n) in the Norwegian wild and semi-domestic reindeer. The allele nomenclature corresponds to* ***Figure 2****. In yellow, the common PRNP allele shared among reindeer, red deer, moose, and roe deer.*

Supplementary material

Supplementary table 1. *Sample sizes (n) from red deer, moose and roe deer per municipality and sorted by counties. The data are consistent with Norway's administrative division valid until January 2020.*

	Municipalities (*n*)		
County	Red deer (105)	Moose (137)	Roe deer (46)
Akershus	Aurskog-Høland (1)	Eidsvoll (1)	Asker (2), Aurskog-Høland (1), Bærum (1), Eidsvoll (2), Gjerdrum (1), Sørum (1), Vestby (2), Ås (1)
Buskerud	Hemsedal (3), Hol (4), Kongsberg (1), Ål (1)	Flå (2), Gol (1), Hemsedal (1), Ål (1)	Hol (1), Hurum (1), Lier (2)
Hedmark	Nord-Odal (1), Rendalen (1), Stor-Elvdal (1)	Elverum (2), Engerdal (3), Os (1), Rendalen (2), Ringsaker (1), Stor-Elvdal (3), Tolga (1), Trysil (2), Åsnes (6)	Løten (2), Nord-Odal (1), Ringsaker (1), Tynset (2), Åsnes (1)
Hordaland	Kvinnherad (2), Voss (14)	-	-
Møre og Romsdal	Stranda (1)	-	-
Oppland	Nord-Fron (4), Søndre Land (1), Vang (1), Vestre Slidre (1), Øyer (4)	Lesja (1), Nord-Aurdal (2), Nord-Fron (1), Ringebu (2), Sel (2), Søndre Land (9), Øyer (1)	-
Rogaland	Karmøy (3), Vindafjord (1)	-	Karmøy (4)
Sogn og Fjordane	Aurland (1), Flora (4), Førde (2), Gloppen (7), Gulen (2), Hornindal (1), Jølster (2), Luster (3), Lærdal (2), Stryn (2), Vågsøy (1), Årdal (5)	-	-
Trøndelag	Hemne (11), Levanger (1), Melhus (3), Midtre Gauldal (1), Namdalseid (1), Namsos (4), Snillfjord (3), Stjørdal (1)	Flatanger (2), Grong (1), Klæbu (1), Namdalseid (1), Namsos (1), Namsskogan (1), Overhalla (6), Selbu (7), Snåase - Snåsa (5), Steinkjer (15), Trondheim (6), Tydal (4)	Hemne (1), Indre Fosen (1), Rennebu (1), Stjørdal (4), Tydal (2)
Vest-Agder	Songdalen (2)		Kvinesdal (2), Mandal (2)
Østfold	Våler (1)	Sarpsborg (1)	Halden (1), Moss (1), Sarpsborg (1), Våler (1)
Aust-Agder	-	Birkenes (1), Froland (1), Åmli (1)	Lillesand (2)
Nordland	-	Bodø (1), Fauske (1), Hattfjelldal (3), Rana (2), Vefsn (5)	-
Troms – Romsa	-	Dyrøy (4), Lenvik (10), Målselv (2), Sørreisa (1), Tromsø (9)	-
Oslo	-		Oslo (1)

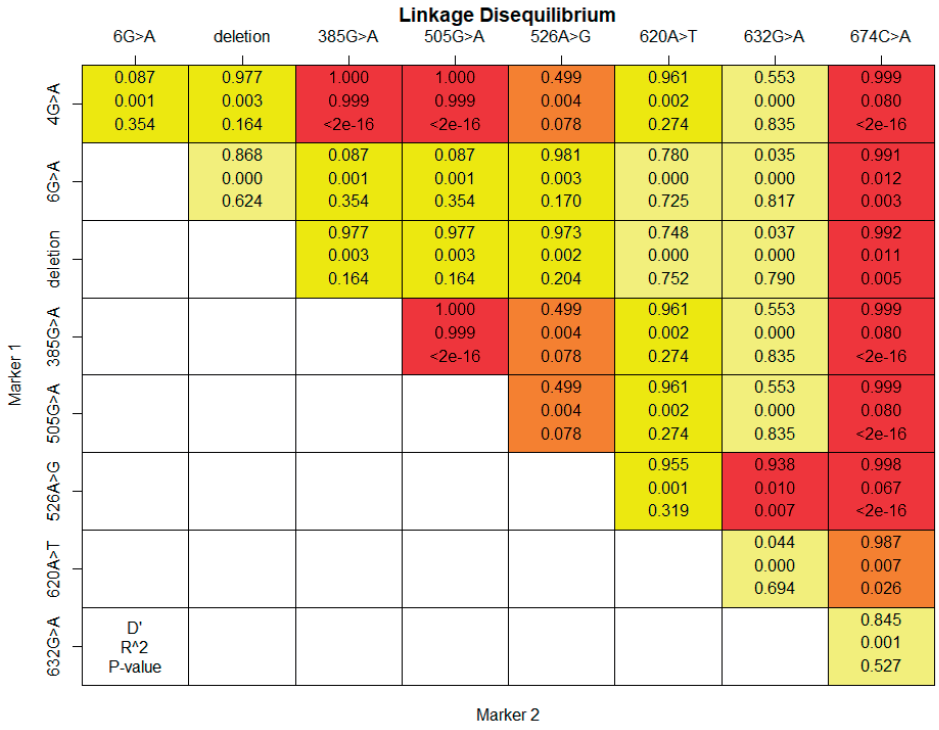
Variant positions in *PRNP*

All four studied species showed variation in *PRNP* coding sequence at the nucleotide level with most variation occurred among reindeer with nine variant positions, followed by red deer with two; whereas both moose and roe deer only showed one (**Supplementary table 2**). The variation consisted of single nucleotide variants (SNVs) encoding both synonymous and non-synonymous substitutions together with an octapeptide repeat deletion limited to reindeer. Novel non-synonymous SNVs included 620A>T and 632G>A in reindeer (Leu207Met and Arg211Gln) linked to 674C (**Supplementary figure 1**). As previously reported in red deer, SNV position 408 is genetically linked to position 676 (D' = 0.999, R^2 = 0.998, P<0.001) indicating 408T linkage with 676C and 408C with 676G.

Supplementary table 2. *Variant positions in PRNP coding sequence from 365 reindeer, 105 red deer, 137 moose and 46 roe deer from Norway. Variant descriptions are in accordance to the HGVS nomenclature definition, using the three-letter amino acid symbol. DNA and predicted protein variation are described based on the correspondent reference sequences for each species. Minor allele frequencies were calculated at the nucleotide level.*

Abbreviations: PRNP=prion protein gene; HGVS=Human Genome Variant Society; DNA=deoxyribonucleic acid

DNA variation	Predicted protein variation	Minor Allele Frequency
Reindeer		
DQ154293.1	**AAZ81474.1**	
4G>A	Val2Met	0.125
6G>A	Val2=	0.022
249_272del	Trp84_Gly91del	0.019
385G>A	Gly129Ser	0.125
505G>A	Val169Met	0.125
526A>G	Asn176Asp	0.107
620A>T	Lys207Met	0.012
632G>A	Arg211Gln	0.001
674C>A	Ser225Tyr	0.359
Red deer		
KT845862.1	**AMP43861.1**	
408T>C	Ala136=	0.890
676C>G	Gln226Glu	0.890
Moose		
MH230115.1	**AZB50215.1**	
325A>C	Lys109Gln	0.226
Roe deer		
MK103016.1	**QAU19526.1**	
72C>T	Cys24=	0.261



Supplementary figure 1. *Pairwise Linkage Disequilibrium (LD) analysis between variant positions (nucleotide) in PRNP coding sequence from reindeer. Each frame summarizes LD between marker 1 and marker 2 measured by D', R^2 and P-value (order shown in the lower left frame). Color-shading based on P-value where red indicates a high significance.*

Abbreviations: LD = linkage disequilibrium; PRNP = prion protein gene; D' = scaled disequilibrium statistic; R^2 = mean squared correlation coefficient

Supplementary table 3. *PRNP alleles in cervid species from Norway. The alleles represent the DNA arrangement within PRNP coding sequence predicted to encode unique prion proteins. Alleles were constructed by phasing non-synonymous variant positions from the study population; thus, alleles harboring only synonymous substitutions (*) were grouped with alleles encoding a similar predicted protein variant i.e. A with A2 and U with U2. Variant descriptions are in accordance to the HGVS nomenclature definition, using the three-letter amino acid symbol. DNA and predicted protein variation are described based on the correspondent reference sequences for each species (refer to* ***Supplementary table 2****).*

Allele	DNA variant	Predicted protein variant
A	DQ154293.1	AAZ81474.1
B	DQ154293.1: c.[674C>A]	AAZ81474.1: p.[(Ser225Tyr)]
C	DQ154293.1: c.[249_272del]	AAZ81474.1: p.[(Trp84_Gly91del)]
D	DQ154293.1: c.[526A>G]	AAZ81474.1: p.[(Asn176Asp)]
E	DQ154293.1: c.[4G>A;385G>A;505G>A]	AAZ81474.1: p.[(Val2Met;Gly129Ser;Val169Met)]
A2*	DQ154293.1: c.[6G>A]	AAZ81474.1: p.[(Val2=)]
F	DQ154293.1: c.[620A>T]	AAZ81474.1: p.[(Lys207Met)]
G	DQ154293.1: c.[632G>A]	AAZ81474.1: p.[(Arg211Gln)]
226Q	KT845862.1	AMP43861.1
226E	KT845862.1: c.[408T>C;676C>G]	AMP43861.1: p.[(Ala136=;Gln226Glu)]
109K	MH230115.1	AZB50215.1
109Q	MH230115.1: c.[325A>C]	AZB50215.1: p.[(Lys109Gln)]
U	MK103016.1	QAU19526.1
U2*	MK103016.1: c.[72C>T]	QAU19526.1: p.[(Cys24=)]

Multinomial model of *PRNP* variability

The predicted probabilities of observing either *PRNP* genotype from the multinomial model in red deer (**Supplementary table 4**) and moose (**Supplementary table 5**) are plotted as an effect of longitude (X) and latitude (Y) (red deer: **Figure 1B, Supplementary figure 2A** and **2B**; moose: **Figure 1C, Supplementary figure 2C** and **2D**). The baseline genotypes were 226Q/226Q in red deer and 109K/109K in moose.

Red deer

The significantly positive estimates for longitude (X) and latitude (Y) indicate a tendency to observe 226E/226Q rather than 226Q/226Q as both X and Y independently increased (**Supplementary table 4**). The same positive tendency applies for 226E/226E compared to 226Q/226Q as longitude (X) increases, but as we move northwards (increase of Y) there was a negative trend for 226E/226E over 226Q/226Q. As X and Y negatively interacted, their effects were weakened at increasing observed values for each other.

Moose

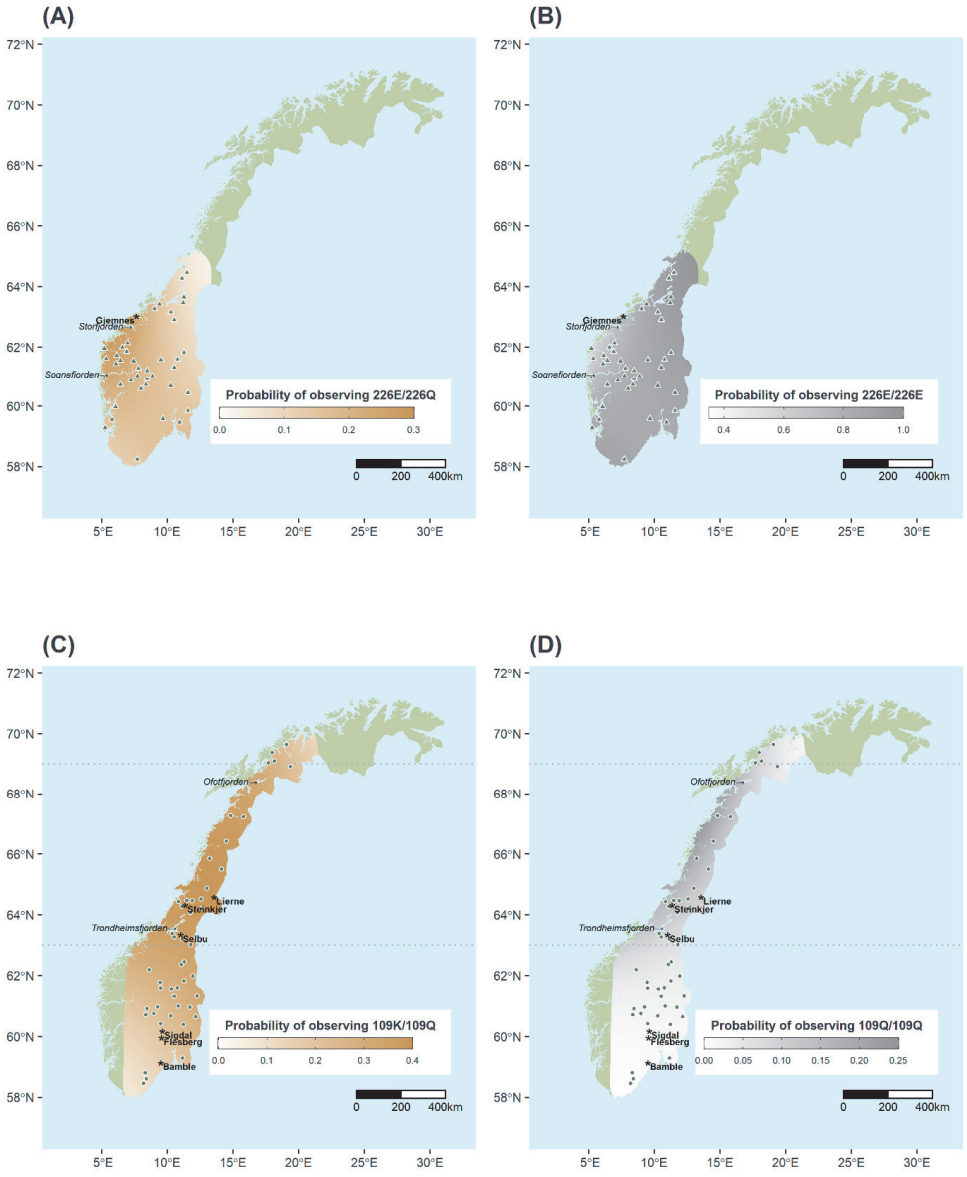
The estimates for both longitude (X) and latitude (Y) were significantly positive, which indicate a trend for either 109K/109Q or 109Q/109Q over 109K/109K as we move to the east (increase of X) or to the north (increase of Y; **Supplementary table 5**). Nonetheless, there was a negative interaction between X and Y, meaning there the positive effect of X was weakened as the observed value for Y increased and vice-versa.

Supplementary table 4. *Estimates from the multinomial regression model of PRNP genotype in Norwegian red deer with X, Y, and their interaction as predictors. Genotype 226Q/226Q was the baseline category.*

Parameter	Estimate	S.E.	Z	*P*	95% CI	
226E/226Q						
Intercept	-18.715	0.090	-208.906	<0.001	-18.891	-18.540
X	13.562	0.821	16.524	<0.001	11.954	15.171
Y	0.203	0.060	3.377	0.001	0.085	0.321
X × Y	-0.199	0.016	-12.314	<0.001	-0.231	-0.168
226E/226E						
Intercept	34.246	0.090	379.636	<0.001	34.069	34.423
X	8.050	0.826	9.744	<0.001	6.431	9.669
Y	-0.661	0.059	-11.192	<0.001	-0.776	-0.545
X × Y	-0.107	0.016	-6.851	<0.001	-0.137	-0.076

Supplementary table 5. *Estimates from the multinomial regression model of PRNP genotype in Norwegian moose with X, Y, and their interaction as predictors. Genotype 109K/109K was the baseline category.*

Parameter	Estimate	S.E.	Z	P	95% CI	
109K/109Q						
Intercept	-45.371	0.059	-765.369	<0.001	-45.487	-45.255
X	2.767	0.815	3.394	0.001	1.169	4.365
Y	0.709	0.037	19.008	<0.001	0.636	0.782
X × Y	-0.044	0.010	-4.367	<0.001	-0.063	-0.024
109Q/109Q						
Intercept	-95.385	0.004	-24825.532	<0.001	-95.392	-95.377
X	4.479	0.052	86.144	<0.001	4.377	4.581
Y	1.503	0.025	61.319	<0.001	1.455	1.551
X × Y	-0.073	0.002	-38.201	<0.001	-0.077	-0.069



Supplementary figure 2. *Spatial interpolation of genotypes distinct from 226Q/226Q in red deer and 109K/109K in moose. Prediction maps for genotypes 226E/Q* ***(A)*** *and 226E/E* ***(B)*** *in red deer, and 109K/109Q* ***(C)*** *and 109Q/Q* ***(D)*** *in moose are based in multinomial logistic regression method. The color bars have been adjusted to better display the range of predicted probabilities for each genotype. The horizontal lines indicate the latitudinal divisions of the Norwegian moose population previously described by Haanes et al. (2011). The sampling sites are respectively represented as triangles for red deer and circles for moose coincident with the centroid of sampled municipalities.*

Haanes, H., Røed, K. H., Solberg, E. J., Herfindal, I., & Sæther, B.-E. (2011). Genetic discontinuities in a continuously distributed and highly mobile ungulate, the Norwegian moose. *Conservation Genetics, 12*(5), 1131. doi:https://doi.org/10.1007/s10592-011-0214-0

Errata list:			
Page	**Line**	**Change from**	**Change to**
v	16	Maria Nöremark Antti Väinö Oksanen	Maria Nöremark, Antti Väinö Oksanen,
6	24	“” slow virus”	“slow virus”
7	6	(Basler et al. 1986)(Basler et al 1986)	(Basler et al. 1986)
54		**Table 4.** *PRNP* genotype (protein level) in Norwegian cervids.	**Table 4.** *PRNP* genotype (protein level) in CWD- affected Norwegian cervids.
57	13	allele E	allele *E*
57	13	allele E	allele *E*

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