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CWD in Norway

**Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee
for Food Safety**

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CWD in Norway.

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Assessed and approved

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

The Norwegian Food Safety Authority (NFSA) and Norwegian Environmental Authority (NEA) asked the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) for an opinion on factors associated with the introduction of Chronic Wasting Disease (CWD) to Norway. VKM appointed a working group consisting of two members of the Panel on Biological Hazards, one member of Panel on Animal Health and Welfare, and two external experts to prepare the answer to the questions. The Panel on Biological Hazards has reviewed and revised the draft prepared by the working group and approved the opinion.

CWD was diagnosed in March 2016 in a wild reindeer (*Rangifer tarandus*) from the Nordfjella mountain area in Norway and in May and June in two mooses (*Alces alces*) in Selbu in South Trøndelag County, approximately 300 km north from the first case.

There is currently no information to determine the origin(s) of CWD agents in Norway. However, the sporadic or genetic (somatic mutation) occurrence of prion disease in cervids cannot be excluded, nor can introduction from North America or other countries. Furthermore, there is no evidence that it has not been circulating at low levels in the Norwegian cervid populations for years, but has not previously been identified. In this scientific opinion, information on prion diseases in general, and CWD in particular, is presented in the light of experiences with this disease in North America.

Prions are among the most resilient pathogens known and dissemination of prions into ecosystems is likely to result in long-term problems. Prions bind strongly to soil and remain infectious. In CWD, prions are present in most peripheral organs and also shed into the environment via saliva, faeces, and urine, as well as with the placenta. CWD transmits easily among cervids, either through direct contact, or indirectly via the environment. Migration of animals is relevant for the spread between areas. Strain diversification might occur in CWD and may influence transmission properties of the agents.

Clinical signs of CWD are non-specific and do not alone enable confirmation of the diagnosis. Analysis of tissue from the brainstem at the level of the obex by approved methods is necessary for diagnosis of CWD. Prion infectivity is assessed by bioassays, often involving transgenic mice. *In vitro* conversion assays, like protein misfolding cyclic amplification (PMCA), provide sensitive quantification of converting activity, which is a good approximation of infectivity.

Genetic variation (polymorphisms) in the gene that encodes PrP (*PRNP*) can modulate sensitivity towards CWD. The level of such genetic variation in Norwegian wild and semi-domesticated cervids is currently unknown.

Cattle and sheep are at very low risk of developing CWD and it is highly unlikely that prion diseases in sheep or cattle are the origin of CWD.

Although transmission of CWD to humans has never been known to occur, and animals other than cervids have not been found to be infected, indicating a species barrier, this possibility cannot be excluded. Thus, measures for reduction of human exposure are recommended. Taking into account uncertainties regarding the plasticity of the CWD agents and the lack of transmission data from the Norwegian isolates, this scientific opinion considers the zoonotic risk of CWD to be very low.

Key words: Cervids, Chronic Wasting Disease (CWD), moose, Norway, prions, reindeer

Sammendrag

Mattilsynet og Miljødirektoratet har bedt Vitenskapskomitéen for mattrygghet (VKM) om å besvare spørsmål knyttet til mattrygghet og dyrehelse etter at den uhelbredelige sykdommen Chronic Wasting Disease (CWD) nylig ble påvist hos en villrein og senere hos to elger i Norge. VKM nedsatte en arbeidsgruppe bestående av to medlemmer fra Faggruppen for hygiene og smittestoffer, ett medlem fra Faggruppen for dyrehelse- og velferd samt to eksterne eksperter, for å utarbeide en vurdering knyttet til de stilte spørsmålene. Faggruppen for hygiene og smittestoffer har lest utkast til rapporten og godkjent vurderingen.

CWD kan ramme og spres mellom en rekke arter av hjortedyr, som rådyr, hjort, elg og reinsdyr. Opprinnelsen til CWD i Norge er ikke kjent. Selv om det aldri er påvist, er det mulig at CWD kan oppstå spontant. Smittestoffet kan også ha kommet til Norge fra andre land. Det er også mulig at CWD har vært i Norge over lengre tid, men på et meget lavt nivå og derfor ikke blitt oppdaget.

I vurderingen gis en generell presentasjon av prionsykdommer, men med vekt på CWD og de erfaringer man har med denne sykdommen i Nord-Amerika.

Smittestoffene som forårsaker prionsykdommene kalles prioner og er blant de mest hardføre vi kjenner og spredning av disse til økosystemer kan medføre langvarige utfordringer. Prionene som forårsaker CWD bindes i jordsmonnet og forblir smittsomme i lang tid.

Når hjortedyr utvikler sykdommen, påvises smittestoffet i mange organer også utenfor sentralnervesystemet og det kan skilles ut i spytt, urin, avføring, morkake og fosterhinner. Det er også påvist smittestoff i hudlaget (basten) som i en periode kler geviret hos hjortedyr. CWD spres mellom hjortedyr, enten gjennom direkte kontakt eller miljøsmitte. Sykdommen kan spres når dyr vandrer eller transporteres mellom områder.

Det er i Nord-Amerika påvist noe variasjon mellom ulike isolater av smittestoffet som forårsaker CWD. Det antas at slik variasjon kan påvirke smittestoffets overføringsevne mellom hjortedyr og muligens evnen til å smitte andre arter, selv om slik smitte ikke er påvist.

De kliniske symptomene ved CWD kan variere og gir alene ikke tilstrekkelig grunnlag for å stille diagnosen. For sikker diagnose må vev fra hjernestammen undersøkes med godkjente metoder.

For å påvise prionsmittestoffet benyttes spesielt følsomme forsøksdyr. For å redusere bruken av forsøksdyr samt gjøre analysene raskere og billigere er det de senere årene utviklet laboratoriemetoder som under optimale betingelser kan gi gode anslag av smittenivået i en prøve. En slik metode kalles PMCA (protein misfolding cyclic amplification).

Arvelige faktorer kan ha betydning for hjortedyrs følsomhet for CWD. Det er kjent at naturlig variasjon i genet som koder for prionproteinet (PRNP) er viktig i denne sammenhengen. Vi kjenner foreløpig ikke nivået av slik variasjon hos norske hjortedyr og man må anta at alle norske hjortedyrarter kan være mottakelige for sykdommen.

Det er svært lav risiko for at sau og storfe rammes av CWD og det regnes som usannsynlig at for eksempel skrapesjuka hos sau er opphavet til CWD.

Det er aldri påvist sykdom hos menneske forårsaket av CWD. Ulike undersøkelser, blant annet med forsøksdyr, tyder på at det er svært liten sannsynlighet for at smittestoffet kan overføres til og gi sykdom hos mennesker. I den foreliggende rapporten anbefales likevel føre-var tiltak som vil begrense menneskers kontakt med smittestoffet.

Background as provided by the Norwegian Food Safety Authority and Norwegian Environment Agency

In April 2016 Chronic Wasting Disease (CWD) was diagnosed by The Norwegian Veterinary Institute in a wild reindeer from the Nordfjella population in the southwest of Norway. A moose was diagnosed with the same disease in May in Trøndelag and another in the same area in June. The disease is recognised as established in North America. However, this is the first detection of CWD in Europe and in wild reindeer worldwide.

Reindeer is a nomadic species that lives in herds, whereas moose is a more solitary animal. Norwegian wild reindeer and moose populations are mainly regulated through harvesting. Modelling effects of different management strategies (e.g. harvesting tactics and the short- and long-term outcome of these) that take into account ecology, demography, and movement of wild reindeer, including scenarios of how the disease spreads, may be relevant in the future.

Due to the lack of information and the lack of experience in handling this disease, the Norwegian Food Safety Authority and the Norwegian Environment Agency hereby requests VKM to provide a scientific opinion.

Terms of reference as provided by the Norwegian Food Safety Authority and Norwegian Environment Agency

Phase 1

1. Food safety

- 1.1. Zoonotic potential – how certain is it that CWD is not a zoonosis?
- 1.2. Is it safe to eat meat (all edible products) from cervids?
 - 1.2.1. Is it safe to eat meat stored in freezers from previous season(s)?
 - 1.2.2. Is it safe to eat meat from animals harvested/slaughtered in the coming season?

1.3. Are there any relevant preventive measures?

1.3.1. If yes, should the measures be applied differently depending on deer species or geographical area?

1.4. Is it safe to eat non-animal products, e.g. berries etc., from the affected area?

1.5. Is it relevant to implement the control or preventive measures established in North America (such as advice for hunters, instructions for carcass handling etc.) in the affected areas in Norway?

2. Disease transmission between animals

2.1. What is the probability of disease transmission between cervids (inter- and intraspecies) in the affected area?

2.2. What are the modes of transmission? (e.g., via urine/faeces, via carcasses or viscera)?

2.2.1. Do CWD prions accumulate in plants/vegetation?

2.3. What is the probability of the disease being transmitted to animals in other geographical areas (directly and indirectly)?

2.4. Which long-term and short-term measures are relevant for preventing disease transmission (e.g. in connection with hunting, field dressing, and handling at slaughterhouse, grazing, farming practices etc.)

2.5. How strong is the species barrier from cervids to other species considered to be?

2.6. Does supplemental feeding (e.g. silage or salt licks) constitute a risk for spreading CWD?

2.7. What are the conditions for wild and semi-domestic cervids in North America vs Norway:

2.7.1. Are there differences that could affect disease transmission between animals (intra/interspecies?) in Norway compared with North America?

2.7.2. Are there any differences in the genotype that could influence an animal's probability of infection (e.g. between wild and semi-domestic reindeer in Norway and between animals in Norway and North America)?

2.8. Is it relevant or necessary to implement the control or preventive measures established in North America (such as movement restrictions etc.) in the affected area in Norway?

Timeframe for phase 1 is 30 June 2016.

Phase 2

1. Food safety

Update of the assessment from phase 1 if necessary

2. Disease transmission between animals

Update of the assessment from phase 1 if necessary

3. The origin of the disease

- a. What is the most likely way the disease occurred in the affected animals (spontaneous mutation, inherited, the known type from North America or a transmission from other species in the area)?

4. Reindeer herding

- a. Which parameters relevant to disease transmission should be included in modelling effects of different management strategies for semi-domesticated reindeer?
- b. Is there knowledge on the sex and age composition of CWD-affected cervid herds in North America that may be relevant to reindeer herding if semi-domesticated reindeer should be affected by CWD?
 - i. Does choice of strategy depend on the occurrence of CWD in a given population?
 - ii. What could be beneficial and non-beneficial side effects of changing the age composition of the herds?

5. Wildlife management

- a. Which parameters relevant to disease transmission should be included in modelling effects of different management strategies if such models should be developed?

- b. Are there any harvesting strategies used in CWD-infected areas in North America that can be relevant for implementation in Norway (e.g. demographic composition)?
 - i. Does choice of strategy depend on the occurrence of CWD in a given population?

Proposed timeframe for phase 2 is to be decided.

Attachment. Export data for the specified products for the last three years, with product breakdown and quantities.

1 Literature

A literature search was performed in PubMed using following search strings:

```
((("prions"[MeSH Terms] OR "prions"[All Fields] OR "prion"[All Fields]) OR ("prion diseases"[MeSH Terms] OR ("prion"[All Fields] AND "diseases"[All Fields]) OR "prion diseases"[All Fields] OR ("prion"[All Fields] AND "disease"[All Fields]) OR "prion disease"[All Fields])) AND cervids[All Fields]
```

Search returned 157 results

```
(CWD[All Fields] OR ("wasting disease, chronic"[MeSH Terms] OR ("wasting"[All Fields] AND "disease"[All Fields] AND "chronic"[All Fields]) OR "chronic wasting disease"[All Fields] OR ("chronic"[All Fields] AND "wasting"[All Fields] AND "disease"[All Fields]))) AND cervids[All Fields]
```

There were no restrictions on date of publication.

The search returned 153 results

Due to time constraints, the literature search could not be exhaustive.

1.1 Relevance screening

The titles of all hits were scanned, and for those that were of potential relevance, the abstracts were also inspected. The relevance screening was performed independently by every member of the working group. Articles were excluded if they did not relate to the terms of reference. The reference lists in selected citations were scrutinized to identify additional articles or reports that had not been identified by the PubMed searches.

2 Prions and prion diseases (hazard identification and characterisation)

In March 2016, chronic wasting disease (CWD) was unexpectedly diagnosed in a wild reindeer (*Rangifer tarandus*) from the Nordfjella mountain area in Norway. This was the first case of CWD diagnosed outside North America and Republic of Korea and the first ever diagnosed in reindeer. Within two months, during the period in which this report was being written, CWD was diagnosed in two moose (*Alces alces*) in Selbu in South Trøndelag County, approximately 300 km north from the first case. All cases were diagnosed by approved methods and the first case has been confirmed by the OIE reference laboratory for CWD in Canada.

Three cases of chronic wasting disease.

Case 1

The first animal, a female reindeer *Rangifer tarandus tarandus*, was found in late March 2016 during field research in Nordfjella by scientists from Norwegian Institute for Nature Research. While using a helicopter to track a free-ranging herd, a sick animal was observed. The animal could not move and died after a short time.

The reindeer was relatively young, estimated age was 3-4 years. The animal was in good condition, about 43 kg. Necropsy showed that the reindeer was not pregnant. Muscular haemorrhages were observed, but no other signs of diseases causing the death of the animal were found.

Case 2

A female moose, *Alces alces*, with abnormal behaviour and in poor condition, was observed in Selbu Municipality in Mid-May 2016. The animal (225 kg) was killed and brought to the nearest veterinary laboratory for necropsy.

The moose was pregnant, estimated age approximately 13 years. The moose was emaciated and had minor injuries.

Case 3

A dead female moose was found in late May in a river in Selbu Municipality, in the same area as Case 2. The animal was in normal condition and weighed 308 kg.

The moose was pregnant with twins. Fracture of one of the ribs and haemothorax was a found at necropsy.

There is currently no information about the source(s) of CWD agents in Norway. The appearance of CWD in Norway represents a turning point in Norwegian wildlife disease surveillance and management, and effects beyond Norwegian borders are possible if the disease is not controlled in Norway.

In this scientific opinion, requested by the Norwegian Food Safety Authority and the Norwegian Environment Agency, information on prion diseases in general, and CWD in particular, is presented in view of experiences with this disease in North America. Populations of cervids in Norway, both semi-domesticated and wild, are also presented before we discuss the probability that CWD will extend into other areas beyond those two already affected. Surveillance data related to the occurrence of prion disease in livestock, semi-domesticated cervids and wild cervids in Norway are also presented. Issues related to food safety, epidemiology, and management are touched upon, but are limited due to lack of data.

2.1 Prions and prion diseases

2.1.1 Prion diseases – background

Prion diseases are fatal neurodegenerative diseases occurring naturally in a limited spectrum of mammals, namely humans and ruminants (Hörnlimann et al., 2006; Prusiner, 1987). However, prion disease has also been diagnosed in captive carnivores, particularly in the order Felidae, after intake of prion-contaminated feedstuffs. This indicates that some carnivores can contract prion disease under natural conditions e.g., after oral intake. Moreover, prion diseases are experimentally transmissible, hence the commonly used term Transmissible Spongiform Encephalopathy (TSE) (Lantos, 1992). Traits common to all human and animal prion diseases include a long incubation period, usually months, dependent on route of transmission, dose, genetic factors, and prion strain (Hörnlimann et al., 2006).

Under experimental conditions, a number of mammalian species are capable of developing prion disease, demonstrating that, at least in theory, these diseases could be present in a wider range of animals than currently acknowledged. Moreover, species that themselves do not develop prion disease might, at least in theory, be silent carriers.

As shown in Table 4 (Appendix I), Creutzfeldt-Jakob disease (CJD) is the dominant human prion disease, and occurs in sporadic, inherited, and infectious modes. All inherited (genetic) forms of CJD, and the rare syndromes Gerstmann-Stäussler-Scheinker disease (GSS) and fatal familial insomnia (FFI), are caused by mutations in the prion protein (PrP)-encoding gene *PRNP*. Inherited prion diseases have been observed only in humans. However, a prominent disease modulatory role of *PRNP* polymorphisms is evident in most ruminant prion diseases (Goldmann, 2008; Goldmann et al., 1990).

Sporadic CJD is the most common human prion disease, with a global occurrence of 1-2 cases per million population annually. This disease is considered to be caused by a rare

event in which the cellular PrP^C spontaneously misfolds into disease-causing conformers known as PrP^{Sc}, although underlying somatic mutations in the *PRNP* gene cannot be ruled out.

Most notable among the human prion diseases is variant CJD (vCJD), which is the only known zoonotic foodborne prion disease (Will et al., 1996). It is commonly accepted that vCJD stems from intake of BSE-contaminated food (Bruce et al., 1997). By June 2016, 230 (3 still alive) cases of vCJD had been verified, of which 178 were in the UK. Considering that millions of humans have been exposed to the BSE agent, the few cases of vCJD clearly indicates a low transmission rate for BSE to humans. Poor transmission of diseases between species is a phenomenon referred to as the **species barrier**.

The most dramatic outbreak of human prion disease was Kuru among the indigenous people of the Foré tribe of Papua New Guinea. The Kuru epidemic reached its peak in early 1960s and nearly wiped out the female population. In a series of pioneering studies, Carleton Gajdusek and Michael Alpers clarified that the Kuru epidemic was fuelled by cannibalistic funeral rituals (Gajdusek et al., 1967). Kuru incubation periods varied from a few years (young children were affected) up to more than 50 years (Collinge et al., 2006).

Human prion diseases are not considered contagious under normal circumstances. However, transmission through surgical procedures, like corneal or dura mater transplants, medical treatment with growth hormone (of human origin) or blood transfusions, illustrates the transmission potential. CJD that is transmitted by medical treatments is known as iatrogenic, iCJD (Prusiner and Hsiao, 1994).

In contrast, epidemiological analyses of some of the ruminant prion diseases reveal a distinct contagious behaviour. This is particularly striking for the spread of classical scrapie among genetically susceptible sheep and for CWD in deer (Williams and Miller, 2003).

For reasons that are poorly understood, in these diseases prion replication occurs actively in peripheral organs, enabling a build-up of infectivity titres and strongly increasing the likelihood of shedding infectious agents to the surroundings. Although not completely clarified nor quantitatively assessed in terms of disease transmission, it has been demonstrated that various bodily secretions and excretions can harbour infectious prion particles, including faeces, urine, saliva, placenta and amniotic fluid, milk, and epidermal debris (skin rubbings) (Gough and Maddison, 2010).

Investigations on a farm affected with sheep scrapie using the highly sensitive method of serial Protein Misfolding Cyclic Amplification (sPMCA) demonstrated that prions were present in airborne dust up to 50 meters from the barns where the animals were kept (Gough et al., 2015). Prions are extremely robust pathogens that will withstand harsh environmental conditions for prolonged periods of time, as well as surviving traditional procedures for disinfection (Hawkins et al., 2015).

Although transmission of prions between different species is normally inefficient, even under experimental conditions, within-species transmission of classical scrapie and CWD is highly efficient among genetically susceptible animals, with attack rates reaching 100 %.

That none of the human prion diseases have the epidemiological behaviour seen in classical scrapie or CWD is fortunate, as such a situation would result in a medical challenge of horrifying proportions.

2.1.2 Nomenclature

The nomenclature of prion diseases and prion agents is inconsistent and has evolved over decades according to their scientific discovery (Prusiner et al., 1998). In most instances, diseases were named long before they were recognized as prion disease or TSE, like CJD, GSS, FFI, Kuru in humans, scrapie in sheep and goats, and CWD in cervids. Some diseases were named after scientists, others descriptively, pointing to aspects of the clinical presentation, such as rubbing and itching in classical scrapie or weight loss (wasting) in CWD. Subsequent discoveries of new disease entities were named as “variants” or “types” of diseases already known (Tranulis et al., 2011) or after the species of occurrence, like BSE. Another example is vCJD, which was a new human prion disease and not merely a variant of CJD. Not surprisingly, the press and the public often confused the different forms and variants of human prion disease because of the improvised nomenclature. In animal prion disease, a similar phenomenon is apparent, with atypical forms of scrapie and BSE, whereas in CWD a sub-division into Type 1 and 2 has been proposed (Angers et al., 2010).

Despite these challenges, elaborate diagnostic criteria involving pathology, host genetics and characterization of protease resistant PrP fragments in western blots (WB) allow accurate diagnostics of known prion diseases in defined hosts, like humans, sheep, cattle or deer.

There is currently no systematic or consistent way of classification of prions as pathogens. It is commonly accepted that prions consist of misfolded aggregates of the host-encoded PrP (Stahl and Prusiner, 1991), but the physiochemical characteristics of these aggregates remain poorly defined (Diaz-Espinoza and Soto, 2012). However, any prion isolate or prion agent will consist of PrP aggregates composed solely of the host's PrP, even if the misfolding process was seeded by PrP aggregates from another species. Upon multiple passage in mice, primary isolates will develop into a so-called mouse-adapted prion isolates. Importantly, these will normally retain structural features derived from the original isolates, thus in prion replication, biological information is transmitted and maintained (stored) solely through protein 3D structure, independent of nucleic acids as molecules of information.

In order to keep track of strains and isolates of prions, scientists in the prion field are concerned with the passage history of any given prion isolate. Generally, disease-associated misfolded PrP is called PrP Scrapie (PrP^{Sc}), regardless of species or disease origin. Some scientists use provisional terms like PrP^{ScJD}, PrP^{FFI}, PrP^{BSE} etc. to identify the source (species

and disease name) of the isolate. Proteinase K-resistant PrP, generated *in vitro*, is known as PrP^{Res}.

As all non-experimental prion diseases in a cervid species should, by definition, be named CWD, the diseased reindeer and moose in Norway suffered from CWD, although a final characterisation of the agent has not been done.

2.1.3 Prions – inactivation and stability

Prion diseases were for many decades assumed to be “slow” viral diseases with long incubation periods (Gajdusek et al., 1967; Hunter, 1972). It was therefore a surprising observation that high doses of ultraviolet radiation, sufficient to inactivate most viruses, did not influence prion infectivity (Alper et al., 1967; Alper et al., 1966). Treatment of prion containing isolates with procedures that denatured proteins, however, totally inactivated all infectivity (Alper et al., 1978). In a large series of studies using experimental prion disease in hamsters, Stanley Prusiner and co-workers managed to purify and characterize the transmission agent. They concluded that it consisted mostly, if not solely, of protein, which was partly protease-resistant (Prusiner et al., 1978). This particle was called proteinaceous infectious particle, subsequently renamed to prion (Prusiner et al., 1982).

It has proven notoriously difficult to achieve detailed structural information about prions (Daus, 2015), but the most infectious particles consist of multimeric protein aggregates enriched in physically stable beta-helical secondary structures. The original notion that prion infectivity consisted of partly proteinase-resistant PrP aggregates has been abandoned since several studies have shown that high prion titres can be found in aggregates largely of proteinase-sensitive PrP conformers (Safar et al., 1998). This is a caveat for the use of proteinase-resistant PrP^{Sc} as a proxy for infectivity. It is a reasonable assumption that a series of equilibria between structural conformers and aggregates of slightly different physiochemical properties might occur, and that certain sub-sets of ill-defined molecular aggregates constitute the “active prion” (i.e., the protein species that actively interacts with a host’s endogenous PrP and misfolds it according to a “template”). With this in mind, it is perhaps unsurprising that identification of “the prion” at a molecular level has proven difficult. Despite these challenges, studies have shown that PrP-aggregates/particles containing around 12 PrP molecules, with a combined molecular mass of about 530 kDa and a particle diameter of 12 nm, are the most infectious fractions. Larger aggregates and fibrillar structures are less infectious. A particle of 12 nm diameter is roughly half the size of the smallest known viruses, like parvovirus with a capsid size of only 18-26 nm (Silveira et al., 2005).

Studies of prion inactivation and disinfection have shown that prions exhibit unusual resistance to conventional physical and chemical procedures, such as autoclaving (121°C for 1h), radiation (UV and ionizing) and a number of chemicals like alcohols and detergents. Autoclaving at higher temperatures and pressure (134°C at 3 atm pressure for 1 h) and chlorine (> 1 ppm) and sodium hydroxide (> 1N) solutions and other chemicals with strong

protein denaturing properties will give significant inactivation of prions (Hörnlimann et al., 2006; Oberthür et al., 2006; Rutala et al., 2010).

Studies have also demonstrated that prion aggregates stick to solid surfaces, for instance steel (like surgical equipment) or soil particles (Johnson et al., 2006b).

2.1.4 Detection of prions – *in vitro* and *in vivo* assays of infectivity and quantification of misfolding capacity

Studies of prion infectivity have been laborious and costly since they have relied upon bioassays of infectivity, e.g. inoculation in laboratory rodents, followed by long incubation periods and comprehensive downstream analysis. Considerable research effort has focused on finding more cost-effective ways of assessing prion infectivity, ideally with a high sensitivity that is comparable to that of bioassays.

It was a major breakthrough when Claudio Soto and collaborators devised an improved system of “prion replication” *in vitro* (Saborio et al., 2001). This method, which was coined protein misfolding cyclic amplification (PMCA), is based on a fundamental idea of prion replication, namely that protein aggregates grow steadily as new proteins are forced into the misfolded state – like building bricks in a Lego structure. Soto assumed that by subjecting large aggregates to an intensive burst of ultrasound (1-2 seconds), they would be partly fragmented and thus give rise to new and smaller aggregates, whereupon subsequent misfolding would occur (Soto et al., 2002). By repeating this (bursts of ultrasound followed by incubation) in many cycles and with an excess of substrate for the process (a source of PrP^C that would usually be fresh brain homogenate), it could be demonstrated that, given the right conditions, a dramatic increase in misfolded PrP conformers would occur. After years of experimentation and optimisation, PMCA is considered a very sensitive assay of prion conversion (Chen et al., 2010). Studies have shown that PMCA can be a powerful method for estimating prion infectivity- In addition, PMCA can be used for exploring strain characteristics (Green et al., 2008b) and model species transmission properties of different isolates (Castilla et al., 2008). Thus, the PMCA method provides a sensitive assay of “prion converting activity”, but has not replaced bioassays for assessment of prion infectivity (Barria et al., 2014b).

Using end-point titrations in the most sensitive bioassays, an infectious unit (IU) sufficient to cause disease in 50 % of the inoculated animals, IU₅₀, has been estimated to be 0.2 attomoles (amol) of PrP (1 amol = 10⁻¹⁸ mol, thus 0.2 amole corresponds to 120 000 molecules). Consequently, methods for measuring prion infectivity should have a sensitivity of around 0.2 amol. Estimates have shown that ELISA detection of prions has a limit varying between 2 000 to 20 000 amol, and western blots down to 200 amol, which are clearly below the desired sensitivity. Under optimal conditions, PMCA can reach a sensitivity of 4 x 10⁻⁵ amol, which corresponds to 26 molecules. PMCA and a modified variant known as serial PMCA (sPMCA) has a sensitivity that is several million times higher than immunoassays and even surpasses bioassays (Chen, Morales et al. 2010). Comparing levels of PrP^{Sc} between

tissues and body fluids using a hamster model and by transforming data presented in Table 1 of Chen et al. (2010) into IU₅₀ units, assuming these to be around 0.2 amol, it can be estimated that one gramme of brain at end-stage would correspond to around 4 billion IU₅₀, spleen 3000 IU₅₀/g, buffy coat 43 IU₅₀/ml, plasma 2.1 IU₅₀/ml and urine 0.03 IU₅₀/ml.

Although these data illustrate huge differences in levels of converting activity between tissues, shedding of prion agents in urine, saliva, and faeces might occur over prolonged periods. In a study of CWD prion excretion in faeces, it was demonstrated that in mule deer the total amount of CWD prions excreted from one animal during the disease period corresponded to the infectivity contained in the whole brain at end-stage disease (Tamguney et al., 2009).

In a recent study, combining bioassay (in hamsters) with PMCA methods, Pritzkow et al showed that prion contamination of grass and soil could result in disease transmission (Pritzkow et al., 2015). It was also observed that grass growing in soil contaminated with prions could take up and transport prions from the soil to the leaves in sufficient amounts to give rise to prion disease after oral challenge, although with low efficiency. Considering the amounts of grass consumed by grazing ruminants and the fact that prions can “survive” and still be infective after more than two years, contamination via grass cannot be ruled out as a transmission route in classical scrapie or CWD. This is also relevant for putative animal-to-human transmission, suggesting that plant material must be considered as a potential vehicle of infection in studies of risk assessment, alongside animal-derived foodstuffs.

Another method, similar to PMCA, for quantification *in vitro* conversion is Quaking-Induced Conversion Assay (QuIC and real-time RT-QuIC), in which vigorous shaking replaces ultrasonic disruption of protein aggregates. By using recombinant PrP as substrate this has been developed into a highly sensitive method that is useful for quantitative analysis (Atarashi et al., 2011; Atarashi et al., 2008).

2.1.5 CWD in North America

In the late 1960s a disease characterised by wasting and gradual development of neurological symptoms was observed in captive mule deer in Colorado. It was initially thought to result from the stress of captivity, intoxication, or nutritional deficiencies. The syndrome was called chronic wasting disease (CWD) and no affected animal would recover from the disease. Further studies by Williams and co-workers at the Department of Veterinary Sciences, University of Wyoming correctly identified CWD in mule deer as a spongiform encephalopathy (Williams and Young, 1980). Shortly afterwards the disease was diagnosed in six captive Rocky Mountain elk (*Cervus canadensis nelsoni*) (Williams and Young, 1982). For the next couple of decades, CWD was considered an exotic disease of local impact, affecting mostly captive deer in north central Colorado and southeastern Wyoming where the disease was recognised as enzootic. In 1996, the disease was observed in a captive elk in Saskatchewan, Canada. The affected animal had been imported from South Dakota and no epidemiological link to Colorado and Wyoming could be established.

Soon afterwards, CWD was recognised in captive elk and subsequently in wild white-tailed deer in South Dakota. The origin of CWD in South Dakota remains unknown. As of today, CWD has been diagnosed in cervids in 22 US states and 2 Canadian provinces. For comprehensive reviews of the history and spread of CWD in North America, see (Gilch et al., 2011; Haley and Hoover, 2015). Until the recent findings of CWD in wild reindeer (*Rangifer tarandus*) and two moose (*Alces alces*) in Norway, CWD had been diagnosed in white-tailed deer (*Odocoileus virginianus*), North American mule deer (*Odocoileus hemionus*), Rocky Mountain elk, and Shira's moose (*Alces alces shiras*), a subspecies of moose (EFSA, 2010).

In some instances, the appearance of CWD in new geographical areas remains enigmatic, as no lateral spread from enzootic zones has been documented. Interestingly, there are apparently unaffected regions located in between affected areas, underlining the discontinuous spread of the disease. We still do not know whether the disease has been widely distributed, but at a low level, and gone unrecognised until picked up through increased awareness and surveillance, or has it spread laterally and silently from defined loci.

2.1.6 Clinical signs of CWD

In CWD, as in other prion diseases, clinical signs result from pathological processes in the central nervous system (CNS) causing neurological disturbances (Williams, 2005). The average incubation period varies from two to four years, and the duration of clinical disease, as observed in captive deer, can vary from a few days to months, even up to one year. However, in most instances death occurs within four months of the onset of clinical disease. It is assumed that survival times will be shorter in free-ranging cervids. Behavioural changes and loss of body condition will progress slowly and, over time, the animals can easily be recognised as diseased, even by untrained observers.

In some instances, animals show hyperexcitability upon handling, and even early clinical CWD cases are susceptible to handling stress, such as from chasing and immobilisation, and show increased post-handling mortality.

In the terminal stages, polydipsia and polyuria might occur and, due to loss of motoric innervation of laryngeal and oesophageal musculature, salivation and dilatation of the oesophagus can be observed. Related to this, aspiration pneumonia is also seen. This might develop at early stages of the disease and cause sudden death. Therefore, in diagnosing aspiration pneumonia in cervids, CWD must be considered (Williams, 2005).

In classical scrapie, pruritus and bilateral hair loss are sometimes the dominant signs, but seem not to be a feature of CWD, although poor body condition will normally be reflected in a rough and dry coat.

One key feature of CWD in cervids is a prolonged process of wasting, i.e. losing energy resources, such as fat, and, later on, also muscle. It is important to keep in mind that both wild and semi-domesticated reindeer, and also other wild cervids, normally lose energy stores and weight during the winter season as a physiological adaptation, unless the animals

are provided with feed during the winter. Thus, during late winter and early spring, reindeer are frequently classified as being in poor condition, but this alone may not be indicative of disease. Moreover, in regions with high animal density for the pasture resources available, and especially after unfavourable winters in which the lichen pastures are ice-covered, emaciation and starvation occur without indicating a state of disease, but rather a prolonged negative energy balance.

Another non-specific characteristic of CWD is the CNS symptoms. There are not many known conditions or diseases in reindeer that are characterised by such symptoms, but the brainworm, *Elaphostrongylus rangiferi*, may cause CNS symptoms when larvae migrate to the CNS where they develop into adult nematodes (3-5 cm), initiating a local immune response. The infection may cause ataxia and paresis of the hind legs. The animal may sometimes sit like a dog, unable to rise, sometimes walk normally, and then again become parietic, and unable to follow the herd. The brainworm is enzootic in both wild and semi-domesticated reindeer in Norway. A study of three wild reindeer populations, indicated that 19 % of the calves and 44 % of adults were infected (Handeland, 2014), whereas an older study in one reindeer herd (Finnmark County, 1976-78) reported a prevalence of 7-68 % in calves (1 year) and 60-100 % in adults (> 3 years), varying between years (Halvorsen et al., 1979). Mostly calves and young animals are affected by elaphostrongylosis, and the prognosis for such animals is usually poor, even with anti-parasitic treatment. Red deer (*Cervus elaphus*) and moose (*Alces alces*) have their own species of brainworms, *E. cervi* and *E. alces*, respectively.

Another condition that may affect CNS is infection with *Listeria monocytogenes*, known to cause CNS symptoms in sheep and goats. *Listeria* has caused bacteraemia in semi-domesticated reindeer, but such infections accompanied by CNS symptoms in reindeer have not been documented. It cannot be ruled out that also other conditions may also cause CNS symptoms, such as insufficiencies and intoxications (e.g. lead poisoning), but these conditions should be regarded as very rare.

2.1.7 Pathology of CWD

The brain areas most heavily affected are in the medulla oblongata and diencephalon. The histopathological features of CWD are similar to those of other ruminant prion diseases, such as classical scrapie (Williams and Young, 1993). In the medulla oblongata, several neuronal nuclei are affected, but most prominently the dorsal nucleus of the vagal nerve. The cerebral cortex and the hippocampus are less heavily affected. One feature of CWD, particularly prominent in white-tailed deer, is the presence of large protein aggregates known as amyloid plaques (Guiroy et al., 1991). When such plaques are surrounded by vacuoles they are known as "florid plaques". Plaque structures have been observed in human prion disease, such as Kuru, in BSE in cattle, and in experimental prion disease in rodents, but they are unusual in classical scrapie. For analysis and screening purposes, samples from medulla oblongata at the level of the obex are considered adequate for CWD diagnosis.

In CWD, PrP^{Sc} aggregates are present in a number of peripheral organs, particularly the lympho-reticular system, including retro-pharyngeal lymph nodes, tonsils and gut-associated lymphoid tissue, spinal cord, and peripheral nerves. Although widespread distribution of PrP^{Sc} in peripheral organs is a prominent feature of CWD, it is not always the case in elk CWD (Race et al., 2007).

Pathology and PrP^{Sc} distribution in experimental CWD in reindeer appears to be similar to that observed in other species, including peripheral distribution of the agent. Two of six reindeer developed CWD after oral inoculation (Mitchell et al., 2012), and the incubation period, clinical presentation, and distribution of PrP^{Sc} (or PrP^{CWD}) provide strong evidence of a similar development of CWD in reindeer as seen in other cervids. Importantly, nearly 100 % of peripheral lymphoid organs were positive for PrP^{Sc}, particularly germinal centres. A large number of other peripheral organs were also positive, such as the pancreas, adrenal glands, thyroid gland, heart, lungs, kidneys, and the urinary bladder, in addition to all segments of the gastro-intestinal tract. No PrP^{Sc} could be detected in the musculoskeletal system by western blot or with the commercially available TSE ELISA kit. As previously noted, these assays are of low sensitivity, and therefore negative findings do not rule out the presence of prions, in, for instance, the musculoskeletal system.

Using highly sensitive mouse bioassays, Angers and co-workers demonstrated a 100 % attack rate in transgenic mice inoculated intra-cerebrally with muscle tissue (semitendinosus and semimembranosus) derived from CWD-affected mule deer. Average incubation times were 426 days, compared with 264 in mice inoculated with CWD from brain (Angers et al., 2006). The authors concluded that muscle might contain CWD infectious agent and that humans consuming or handling meat from CWD-infected deer might be exposed.

In another study, Daus and collaborators (Daus et al., 2011) demonstrated PrP^{Sc} in skeletal muscle from white-tailed deer with CWD using western blots. Analysis of converting activity by PMCA indicated that levels of misfolded PrP in skeletal muscle were 2 000 – 10 000 fold lower than in brain tissue. In muscle, PrP^{Sc} was not found in myocytes (muscle cells), but in intramuscular nervous tissue. The authors concluded that precautionary measures for food safety should be taken to prevent CWD-infected material entering the human diet.

2.1.8 Genetics and CWD susceptibility

Similar to observations made on classical and atypical/Nor98 scrapie in sheep and goats, disease susceptibility to CWD is modulated by single-point mutations in *PRNP*. It is important to keep in mind that in discussing susceptibility and resistance towards prion disease, data suggest that although some genetic variants clearly are protective, there is no evidence of absolute resistance. More often it is a question of prolonging the incubation period.

In white-tailed deer (*Odocoileus virginianus*) disease-modulating polymorphisms in *PRNP* have been detected at positions Q95H, G96S, and S116G (Johnson et al., 2006a), in mule deer (*Odocoileus hemionus*) at codon S225F (Jewell et al., 2005), and in Rocky Mountain elk

(*Cervus canadensis nelsoni*) at M132L (corresponding to the human M129L) (Green et al., 2008a). In red deer, (*Cervus elaphus*) polymorphisms leading to amino acid substitutions have been observed at codons G59S, T98A, P168S, and Q225E (Peletto et al., 2009). In moose (*Alces alces*), a polymorphism at codon K109Q has been described (Wik et al., 2012).

There is limited information about PrP genetic variation in reindeer. One study (Happ et al., 2007) reported *PRNP* polymorphisms in caribou (*Rangifer tarandus granti*) from three regions in Alaska. In a total sample of 78 animals, they observed single-nucleotide polymorphisms (SNPs) at five locations, leading to the following amino-acid substitutions in PrP: codon 2 V/M, codon 129 G/S, codon 138 S/N, codon 169 V/M. The fifth SNP was a synonymous variation at nucleotide 438, corresponding to codon 146 N/N. The following PrP proteins were encoded: V2G129N138V169 (short form VGNV), VGSV, VSSV, and MSSM. The allele frequencies were VGSV (about 64 %), VGNV (30 %), VSSV (2 %), and MSSM (4 %). The potential relevance of these data for CWD susceptibility in reindeer was illustrated in a study in which 6 reindeer (*Rangifer tarandus tarandus*) were subjected to oral challenge with CWD derived from either elk (3 reindeer) or white-tailed deer (3 reindeer) (Mitchell et al., 2012). PrP genetic analysis of the recipient reindeer revealed these PrP genotypes: VV2GG129SN138VV169 (2 animals) VM2GS129SN138VM169 (2 animals) and VV2GG129SS138VV169 (2 animals). The two latter animals, being homozygous SS138, were positive using rectal biopsy after 13 months and developed clinical CWD by 18 months. Duration of clinical disease was 1-2 months. No other reindeer developed CWD or had positive tests by rectal biopsy after more than 22 months (one animal at 61 months) post inoculation. Both diseased animals were inoculated with a CWD isolate from white-tailed deer and the authors discussed whether this inoculum could be more virulent for reindeer than the one from elk, but conclude that this is unlikely. Rather, they focused on the importance of PrP genetic variation at codon 138 S -> N as the major factor in explaining the results, arguing that being homozygous SS138 increases CWD susceptibility compared with heterozygous SN138 (no reindeer in their sample were homozygous NN138). Interestingly, in fallow deer, N138 is present in PrP and in a study that involved 41 fallow deer that were exposed to CWD by being housed together with CWD-affected mule deer, none of the fallow deer developed CWD, even after 7 years (Rhyan et al., 2011). Taken together, these and other data suggest that the presence of N138 reduces CWD susceptibility in reindeer and fallow deer. However, further studies are needed to confirm this.

In the above-mentioned study of caribou, N138 was present in about 50 % of the animals and in accordance with the Hardy-Weinberg principle (Happ et al., 2007).

In a study that included seven semi-domesticated reindeer from Finnmark County, one wild reindeer from the Hardangervidda mountain plateau, and one reindeer from the Svalbard archipelago, a previously unrecognised polymorphism at codon K109D was observed (Wik et al., 2012).

2.1.9 Experimental transmission of CWD and assessment of zoonotic potential

Assessment of the zoonotic potential of a prion disease will normally rely on combinations of epidemiological data, bioassays (natural and transgenic hosts), and *in vitro* modelling of converting activity by PMCA and variants thereof.

CJD surveillance in CWD areas has been particularly vigilant concerning occurrence of CJD cases with unusual disease phenotypes, such as young age. Five cases have been identified and analysed in detail. The results of these investigations provided diagnoses such as genetic CJD, early-onset Alzheimer's disease (Anderson et al., 2007), and sporadic CJD (Belay et al., 2001).

Experiments have shown that intracerebral inoculation is more efficient for transmission than oral inoculation, and that secondary transmission is generally more efficient than primary transmission. The clinical and "real-life" relevance of intracerebral inoculation has been questioned, arguing that oral inoculation provides data of higher relevance, even if oral inoculation often involves large doses of highly infectious material being introduced into the gastro-intestinal tract. This should be borne in mind when evaluating cross-species transmission of CWD and other prion diseases.

CWD has been experimentally transmitted through intracerebral inoculation to a large number of different mammals, including sheep (Hamir et al., 2006a), goats (Williams and Young, 1992), cattle (Hamir et al., 2006b; Hamir et al., 2007), North American non-laboratory rodents (deer mice, meadow voles, white-footed mouse and red-backed voles) (Heisey et al., 2010), cat (Mathiason et al., 2013), ferret (Sigurdson et al., 2008), and non-human primates, like squirrel monkey (Marsh et al., 2005; Race et al., 2014).

The initial transmission of CWD to squirrel monkey (*Saimiri sciureus*) reported by Marsh and collaborators (Marsh et al., 2005), inspired a broader study in which intracerebral and oral inoculation were compared and also involved cynomolgus macaques (*Macaca fascicularis*) that are more closely related to humans (Race et al., 2009). This study confirmed the susceptibility of squirrel monkey to CWD after intracerebral inoculation, with 80 % of the animals developing clinical signs, whereas after oral exposure 15 % of the squirrel monkeys developed disease. None of the macaques developed prion disease, regardless of inoculation route, and remained healthy 70 months after inoculation.

Subsequently, it was observed that upon secondary transmission of CWD in squirrel monkey, the incubation period decreased by more than 11 months compared with first passage, demonstrating the species barrier. Interestingly, inoculation of the squirrel-adapted CWD prion failed to produce disease in macaques after more than 72 months incubation (Race et al., 2014). Furthermore, analysis of the macaques inoculated in the original study revealed no sign of prion disease more than 10 years post inoculation. The authors concluded that these data and results from studies with transgenic mice and *in vitro* experiments demonstrate that humans are at low risk of contracting CWD.

One challenge in utilising prion disease bioassays, particularly in mice, is the prolonged preclinical incubation period, which can extend beyond the normal lifespan of the animal. A reminder of this was provided by Comoy and collaborators (Comoy et al., 2015) demonstrating that after intracerebral inoculation of a cynomolgus macaque with a prion isolate from classical scrapie, the animal developed prion disease after a 10-year silent incubation period. The authors concluded that long-term experimental challenge studies would improve assessment of the zoonotic potential of animal prion disease, not least CWD. Such studies are, however, extremely costly, and with incubation periods extending more than 10 years, inherently slow.

The PMCA method has been used to compare the zoonotic potential of animal prion disease, including BSE, atypical BSE, classical scrapie and atypical/Nor98, and CWD (Barria et al., 2014a). In that study, classical BSE prions proved most efficient at converting human PrP, whereas atypical BSE and all forms of scrapie failed to convert human PrP. However, CWD prions converted human PrP under the influence of PrP-genetic modulation, with homozygosity MM at codon 129 (in human PrP) as most efficient. Interestingly, characterisation of the PrP^{Res} material generated showed physicochemical properties similar to the most common sporadic form of CJD, known as Type 1 MM1 sCJD. The zoonotic and diagnostic implications of these data are further presented and discussed by Barria et al. (Barria et al., 2014b). Although modelling of *in vitro* conversion of PrP from different species, under non-denaturing or denaturing conditions, provides valuable data and insights into the molecular nature of the species barrier, translation of such data into quantitative assessment of transmission properties between species remains a crude approximation. In the above-mentioned study by Barria et al., conversion properties of animal prion isolates were compared with that of classical BSE (Barria et al., 2014a). This approach provides some calibration of the animal prion isolates, since, in the case of classical BSE, epidemiological data are available that provide estimates of human oral susceptibility, which must be considered very low. They observed conversion of human PrP by CWD, but with less efficiency than that of classical BSE, suggesting a lower susceptibility.

Transgenic mice overexpressing the human PrP (“humanised mice”) were not susceptible to CWD after intracerebral inoculation, indicating low zoonotic potential (Sandberg et al., 2010). Further studies of this kind are in progress and the results will provide valuable information for further assessment of the zoonotic potential of CWD prions.

2.1.9.1 Transmission of CWD to livestock

Among cervids, CWD appears to spread easily, apparently with little or no species barriers. In contrast, several studies have shown that cattle and sheep fail to develop CWD upon oral challenge and, in the case of sheep, only after very long incubation periods following intracerebral inoculation. Considering oral infection, which is most relevant, these data argue that transmission of CWD to grazing sheep or cattle seems unlikely (Hamir et al., 2011; Hamir et al., 2005; Hamir et al., 2007). These data also demonstrate that it is highly unlikely

that CWD originated as a disease of cattle or sheep, since reintroduction of the agent to the original host species would be expected to be efficient, but this is not observed.

2.1.9.2 The CWD agent is not one single, stable entity

It is well established that prion agents can diversify into variants, called strains, and that several strains can be present in a host simultaneously; there is ample evidence to suggest that at least two strains of CWD exist (Angers et al., 2010). When a prion agent replicates in a new host (species), new strain characteristics might develop. Moreover, physiochemical alterations of the agent might also occur under abiotic and biotic environmental exposures, which might constitute a strain selection pressure. For a discussion of prion strains and their plasticity in relation to species barrier see (Beringue et al., 2008).

It should be noted that CWD strains can have varied zoonotic potential and we currently lack data on transmission properties of the Norwegian CWD isolates.

2.1.10 Environmental spread and persistence of CWD prions

Efficient spread into the environment of robust pathogens that transmit effectively to susceptible animals poses significant challenges for disease management. A further complicating issue is that there are currently no efficient ways of identifying asymptomatic carriers that shed the pathogenic agent.

Controlling or eradicating CWD in captive herds of deer in North America has proven difficult despite massive efforts, and many scientists consider this task for populations of wild cervids to be even more problematic, if not impossible. In New York State, however, intensive deer depopulation combined with surveillance has proven efficient in eliminating CWD, and the same approach has also been used successfully in southwest Minnesota (Saunders et al., 2012). Eradication and control efforts are more likely to succeed if initiated at an early stage of an epizootic, before the disease reaches enzootic proportions, after which eradication will be almost impossible. This is also relevant for the herds of semi-domesticated reindeer and for maintaining reindeer herding as a viable industry.

CWD prions might enter the environment via carcass decomposition (Miller et al., 2004), antler velvet and skin (Angers et al., 2009), saliva (Mathiason et al., 2006), urine (Haley et al., 2009), faeces (Safar et al., 2008; Tamguney et al., 2009), and most probably via placenta and milk, as shown with classical scrapie (Andreoletti et al., 2002; Moore et al., 2008).

It is well documented that prions adsorb to soil components (Cooke et al., 2007; Genovesi et al., 2007; Ma et al., 2007) and remain infectious for prolonged periods of time (Johnson et al., 2006b; Seidel et al., 2007). Soils are extremely diverse and data suggest that prions bind more tightly to clay components, which might even enhance their infectious potential as compared with prions bound to other soil components (Kuznetsova et al., 2014). It is

reasonable to assume that the tight binding of prions to soil components will limit further distribution, although plant roots might take up prions from the soil and transport these to their leaves. This also suggests that prions will be bound to the upper few centimetres of soil and thus be readily available for plants, but also exposed to overland flow, for instance during heavy rainfall or snow melting, with subsequent run-off to water bodies (Nichols et al., 2009). By using sPMCA methods, CWD prions were detected in one environmental sample (water), although below infectious levels (Nichols et al., 2009).

In reviewing prion transmission, Gough and Maddison (2010) presented a tentative listing of infectivity levels, based upon data from sheep scrapie and CWD: in descending order: **placenta>skin (and antler)>milk>faeces>urine>saliva>soil>water**. In addition, material from a carcass, including CNS and gastro-intestinal tract, should be considered highly infectious, based upon distribution of the agent. It should also be noted that repeated exposure to materials like saliva and urine might be biologically important, despite relatively low infectivity. Prion infectivity in saliva is also important because very large volumes of saliva are swallowed daily. This will potentially re-infect the gastro-intestinal tract and probably increase faecal excretion.

Some rodent species are capable of developing CWD prion disease after intra-cerebral inoculation and oral intake. Thus, wild rodents should be considered as a potential reservoir of prions. Moreover, a recent study showed that CWD prions remained infectious after passage through the digestive system of coyotes (Nichols et al., 2015).

In Norway, many species of rodents and carnivores may contribute to the dissemination of CWD prions from a CWD carcass. These include: Norway lemming (*Lemmus lemmus*), tundra vole (*Microtus oeconomus*), bank vole (*Myodes glareolus*), gray-sided vole (*Myodes rufocanus*), field vole (*Microtus agrestis*), long-tailed field mouse/wood mouse (*Apodemus sylvaticus*), squirrel (*Sciurus vulgaris*), wolverine (*Gulo gulo*), wolf (*Canis lupus*), brown bear (*Ursus arctos*), Arctic fox (*Vulpes lagopus*), marten (*Martes martes*), weasel (*Mustela erminea*), and the least weasel (*Mustela nivalis*), as well as many species of birds of prey (owls, eagles, hawks, and falcons) and scavenger birds, such as crows and ravens.

2.1.11 Summary on prions and CWD

Prions are among the most resilient pathogens known. Dissemination of prions into ecosystems may result in long-term problems. Prions bind strongly to soil and remain infectious.

In CWD, infectivity is present in most peripheral organs and also shed into the environment via saliva, faeces, and urine, as well as with placenta.

Plants may absorb infectivity from soil, and CWD has been transmitted to laboratory rodents via plant material.

CWD transmits easily among cervids, through direct contact or indirectly via the environment. Migration of animals contributes to the spread between areas, which is highly relevant for both wild cervids and semi-domesticated reindeer.

Genetic variation (polymorphisms) in the gene that encodes for PrP (*PRNP*) can modulate sensitivity towards CWD. The level of such genetic variation in Norwegian wild and semi-domesticated cervids is currently unknown.

The human species barrier towards CWD prions is very strong. Although transmission of CWD to humans has not been known to occur, this cannot be excluded.

Measures for reduction of human exposure should be implemented.

Cattle and sheep are at very low risk of developing CWD, and it is highly unlikely that prion diseases in sheep or cattle are the origin of CWD.

Sporadic or genetic (somatic mutation) occurrence of prion disease in cervids cannot be ruled out.

Strain diversification might occur in CWD. This may influence transmission properties.

Clinical signs of CWD are non-specific and do not alone allow confirmation of diagnosis.

Analysis of tissue from the brainstem at the level of the obex by approved methods is currently necessary for diagnosis of CWD.

Prion infectivity is assessed by bioassays, often involving transgenic mice.

In vitro conversion assays, like PMCA, provide sensitive quantification of converting activity, a good approximation of infectivity.

Assessment of zoonotic potential of animal prion diseases combines epidemiological and experimental data sets.

Large-scale screening of wild and semi-domesticated cervids in Norway is needed to map the occurrence of CWD.

2.2 Cervids in Norway

Deer species (cervids) of the family Cervidae have played important roles throughout human history, as an important source of meat and materials, such as skin and antlers, as reflected in mythology, religion, and cultural expression. Cervids are ruminant plant eaters, of which some mostly feed on plants on the ground (grazers), whereas others are browsers, finding most of their food in trees and bushes. They give birth to one or two calves each year, typically in the spring. Some species live as solitary animals, whereas others, such as

reindeer, are gregarious animals, living in larger herds, and usually conducting seasonal migrations between summer and winter pastures.

Within the family Cervidae the subfamily Cervinae includes the red deer (No: "hjort" or "kronhjort", Lat: *Cervus elaphus*) fallow deer (No: dâhjort or "dâdyr", Lat: *Dama dama*), while the subfamily Capreolinae includes the reindeer (No: "reinsdyr", Lat: *Rangifer tarandus*), which is commonly called caribou in North America, roe deer (No: "rådyr", Lat: *Capreolus capreolus*) and moose/Eurasian elk (No: "elg", Lat: *Alces alces*).

2.2.1 Reindeer

Reindeer are grey to brown in colour and, in contrast to other cervids, both sexes have antlers which are shed after the rut in the fall (males) or later during winter and spring (females). Reindeer give birth to one calf (twin calves are extremely rare) in late April to early June. They become sexually mature at approximately 1.5 years of age, but this is, among other factors, dependent on body size, and occasionally female calves born in spring give birth as a one year old. Reindeer vary considerably in size, both between and within the different subspecies.

2.2.1.1 Reindeer subspecies and distribution

Reindeer, commonly called caribou in North America, all belong to the same species, *Rangifer tarandus*. However, about 55 species and subspecies have previously been described, of which some are now extinct. Banfield's revision and summary of the classification of reindeer and caribou (Banfield, 1961) is now widely accepted, with seven subspecies (Figure 2-1):

1. Eurasian tundra reindeer (*R. t. tarandus*) is almost continuously distributed in Eurasia, across the tundra region, including the treelines and mountain areas. In parts of its distribution, this subspecies may overlap with the Eurasian forest reindeer (see below).
2. Eurasian forest reindeer (*R. t. fennicus*) was primarily distributed in the border region between Finland and Russia, but it remains unresolved whether reindeer from the forest regions in other areas in northern Eurasia should be included within the same subspecies.
3. Alaska tundra caribou (*R. t. granti*), also called Grant's reindeer, is distributed in most parts of the Alaska Peninsula and into the Yukon Territory, Canada.
4. Canadian barrenground caribou (*R. t. groenlandicus*) is also distributed in the tundra region in North West Territories, Canada. Also, the caribou on Baffin Island and the western part of Greenland are included within this subspecies.
5. American woodland caribou (*R. t. caribou*) is distributed in the woodland area south to the barrenground caribou, from a few localities in the Yukon Territories in the west to New Foundland in the east, including the large herds in the Quebec region.

6. Peary caribou (*R. t. pearyi*) lives on the Arctic Islands of northern Canada.
7. Svalbard reindeer (*R. t. platyrhynchus*) inhabit the Svalbard archipelago. The Peary caribou and the Svalbard reindeer together constitute the Arctic ecotype with short legs, pale and highly insular pelage, small ears and a short rostrum.

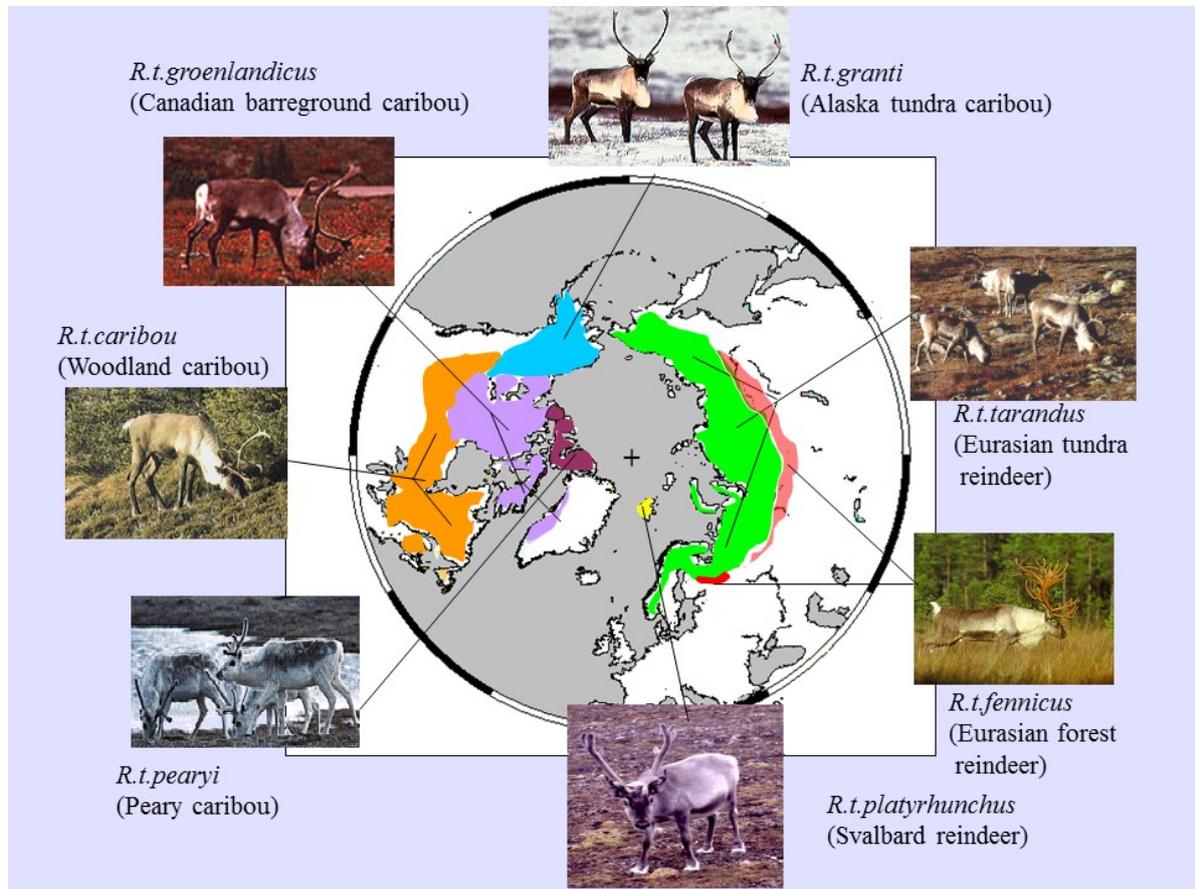


Figure 2-1. Distribution of the seven subspecies of reindeer (*Rangifer tarandus*) (Knut Røed, NVH/NMBU).

2.2.1.2 Wild and semi-domesticated reindeer

In mainland Norway, all reindeer belong to the same subspecies, the Eurasian tundra reindeer, *R. t. tarandus*. Reindeer were previously distributed throughout large regions of Eurasia, migrating north as the retreating glaciers uncovered the landmasses at the end of the last ice age. Today, the 23 **wild** reindeer populations in southern Norway are regarded as the remaining wild reindeer of this species (Figure 2-2). Except for a few smaller regions in Finland inhabited by wild Eurasian forest reindeer, all other reindeer in Fennoscandia belong to the Eurasian tundra reindeer subspecies and are herded. These animals are thus not wild, but they are not regarded as domesticated either, compared with cattle, sheep, and goats. Herded reindeer (i.e. owned by someone) are therefore commonly referred to as **semi-domesticated**. In Norway and Sweden, reindeer herding is a traditional cornerstone

of the Sami culture, and, with a few exceptions, only people with a Sami heritage can own reindeer. In Finland, there are both Sami and non-Sami reindeer herders.

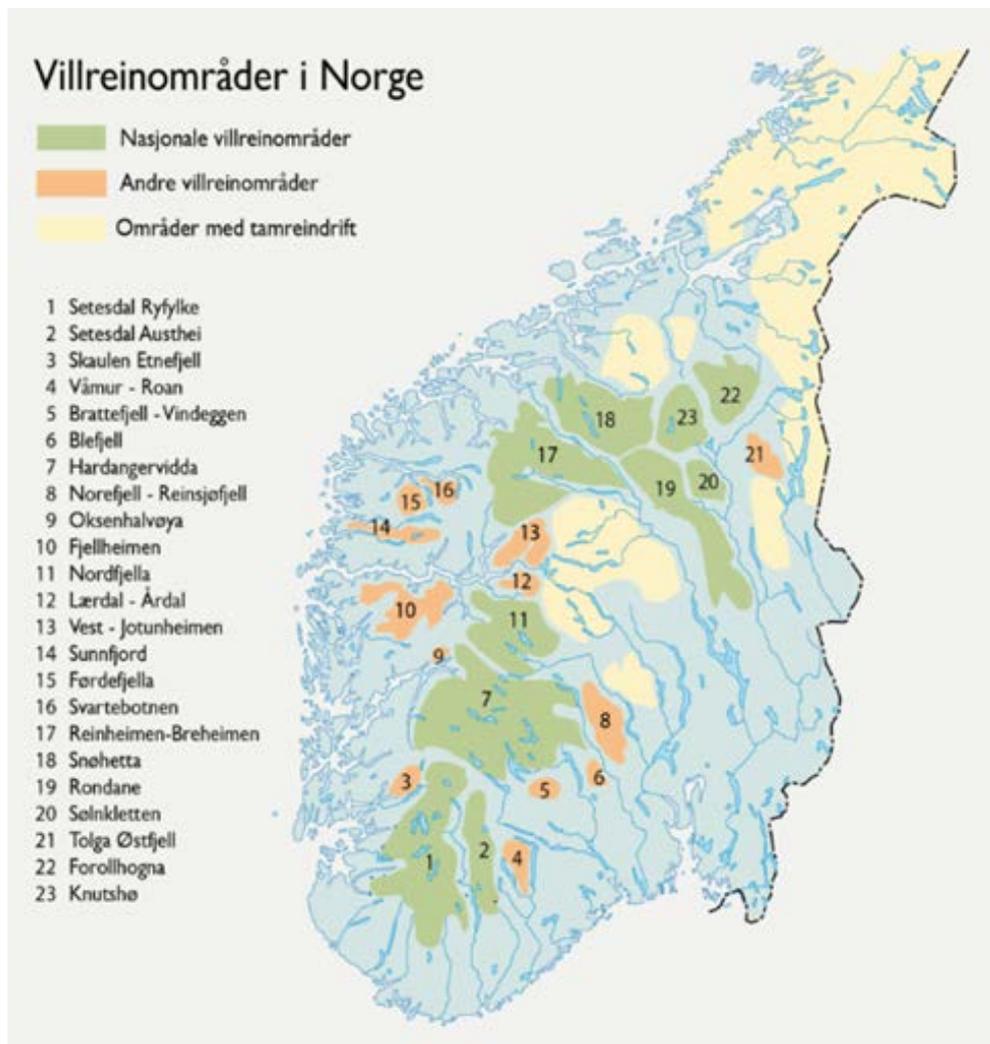


Figure 2-2. Distribution of the 23 different populations of wild Eurasian reindeer (*R. t. tarandus*) in Norway. (<http://www.villrein.no/om-villreinomrdene>). (Nordfjella, region 11).

2.2.1.3 Wild reindeer populations in Norway

Wild reindeer live mostly in high mountain areas, but in certain regions, forest habitats are also important. The body length may reach 220 cm from nose to tail, a wither height of 125 cm, and a body weight of 270 kg for large males, whereas females are considerably smaller. In winter, as much as 40-80 % of the food are lichens (*Cladina* spp.), whereas in summer they eat grass, sedges, herbs, and some shoots and leaves, depending on the habitat and what is available. Wild reindeer usually migrate on a seasonal basis between summer and winter pastures, although infrastructure, such as roads and railways, and natural boundaries, such as rivers, lakes, and valleys, may limit migration. Furthermore, some of the wild reindeer populations have only a very restricted area, not allowing seasonal migration. It is

also a matter of dispute and scientific investigation whether wild reindeer populations all represent the original wild reindeer population, or whether they have been genetically mixed with semi-domesticated reindeer through interbreeding to varying degrees.

The status and development of the 23 different wild reindeer populations in Norway vary, but the total harvest has increased, from 4778 animals in 2010 to 6507 animals in 2015 (mean 6189), with the highest number for 2014 (7944) (www.hjorteviltregisteret.no).

The wild reindeer in Nordfjella (CWD case 1)

Nordfjella (Figure 2-2, region 11) was previously a part of a larger habitat for wild reindeer, stretching from Jotunheimen and Valdres in the north, to Hardangervidda and Setesdalsheiane in the south. The Nordfjella wild reindeer are now distributed over a 2 995 km² region north of the Hardangervidda mountain plateau; from the railway (Bergensbanen) in the south to the road Riksveg 52 (Gol – Borlaug) in the north, and within the natural limitations of Aurland/Aurlandsfjorden in the west, Lærdal/Sognefjorden in northwest and the valley Hemsedal – Hol – Ål in the east (Figure 2-3). This region is divided into two management regions, Forvaltningszone I and II (Management zone I and II). A large part of the Nordfjella area is high alpine mountainous regions, including high mountain peaks and ridges, deep valleys, and glaciers. The population is currently estimated to be approximately 2 500 animals.

Historically, during the 18th and 19th centuries and into the first half of the 20th century, reindeer were herded in several regions of what today is defined as the Nordfjella wild reindeer region, with frequent mingling between wild reindeer and semi-domesticated reindeer. The Second World War (1940 – 1945) contributed to a shift from reindeer herding to management of the wild reindeer, but reindeer herding (Østre Hol Tamreinlag) continued until the beginning of the 1980s.

After 2006, it has been shown that reindeer have migrated from Hardangervidda in the south into Nordfjella, that approximately 1000 reindeer from southern parts of Nordfjella (Forvaltningsområde II) annually have used summer pastures south of the Bergensbanen railway, and that a herd of approximately 100 animals have used pastures west of Hardangerjøkulen glacier during winter, indicating contact between the Nordfjella and other reindeer populations (Strand et al., 2011).



Figure 2-3. Nordfjella region, wild reindeer area surrounded by black line.

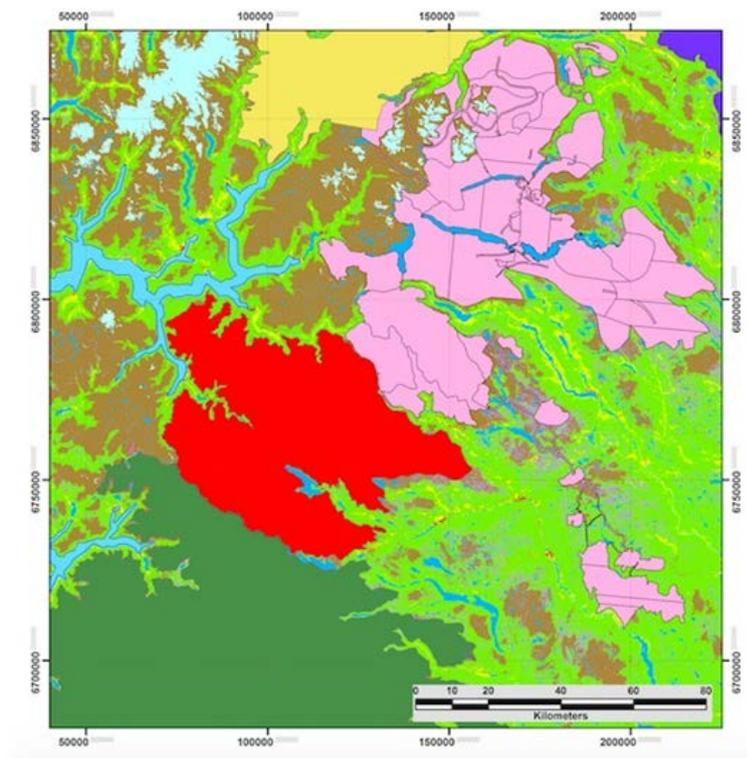


Figure 2-4. Distribution of the Nordfjella wild reindeer population (CWD case 1) (red), and the border with the Hardangervidda wild reindeer population (dark green) and the neighbouring district with semi-domesticated reindeer (pink). (Map: Bernt Johansen, NORUT).

The Nordfjella wild reindeer management region (Figure 2-3, Figure 2-4, red) borders the region Hemsedalsfjella mountains, north of the road Riksveg 52, and is a part of the non-Sami reindeer herding, i.e. "tamreinlagene" (Figure 2-4, pink). There is still some contact between the Nordfjella wild reindeer and the semi-domesticated reindeer in the north, the latter occasionally entering the Nordfjella management zones (Strand et al., 2011).

During the last six years, the harvest of reindeer from the Nordfjella population has varied from 266 animals (2012) to 522 animals (2014), with a mean number of 448 animals during 2010 – 2015 (www.hjorteviltregisteret.no).

Some of the wild reindeer populations, but not the Nordfjella population, are included in the national surveillance programme for deer species (Det nasjonale overvåkingsprogrammet for hjortevilt) run by the Norwegian Institute for Nature Research (NINA). Some investigations on health and diseases have been conducted through the surveillance programme for deer health (Helseovervåkingsprogrammet for hjortevilt; HOP) run by the Norwegian Veterinary Institute (NVI). In general, infections with alphaherpes- and pestivirus as well as the brainworm (*Elaphostrongylus rangiferi*), the lungworm (*Dictyocaulus eckerti*), the larval stages of the warble fly (*Hypoderma tarandi*), and throat bot (*Cephenemyia trompe*) are considered common. There are also cases of papillomatosis (papillomavirus), and one recorded outbreak of digital necrobacillosis (*Fusobacterium necrobacillosis*) (Handeland et al., 2010), as well as sporadic findings of pasteurellosis and other bacterial and parasitic infections and infestations (Handeland, 2014). There are no previous reports on diseased reindeer with CNS symptoms from the Nordfjella population.

2.2.1.4 Semi-domesticated reindeer and reindeer herding in Norway

Reindeer herding is conducted in 140 municipalities in Norway, using around 140 000 km² or about 40 % of the landmass of Norway. Reindeer herding is organised in six reindeer pasture regions (Øst-Finnmark, Vest-Finnmark, Troms, Nordland, Nord-Trøndelag and Sør-Trøndelag) with approximately 80 herding districts, each consisting of several reindeer herding units (siida) for summer and winter pastures, respectively (Figure 2-5). The herding usually has a semi-nomadic structure, in which animals are herded or transported (by truck or boat) between winter pastures and the calving ground/summer pastures. Reindeer feed on many different plants during the summer, depending on the local habitats and resources, which support most of the reproduction, whereas the winter pastures, often high mountain plateaus such as Finnmarksvidda, are regarded as maintenance feed (for winter survival).

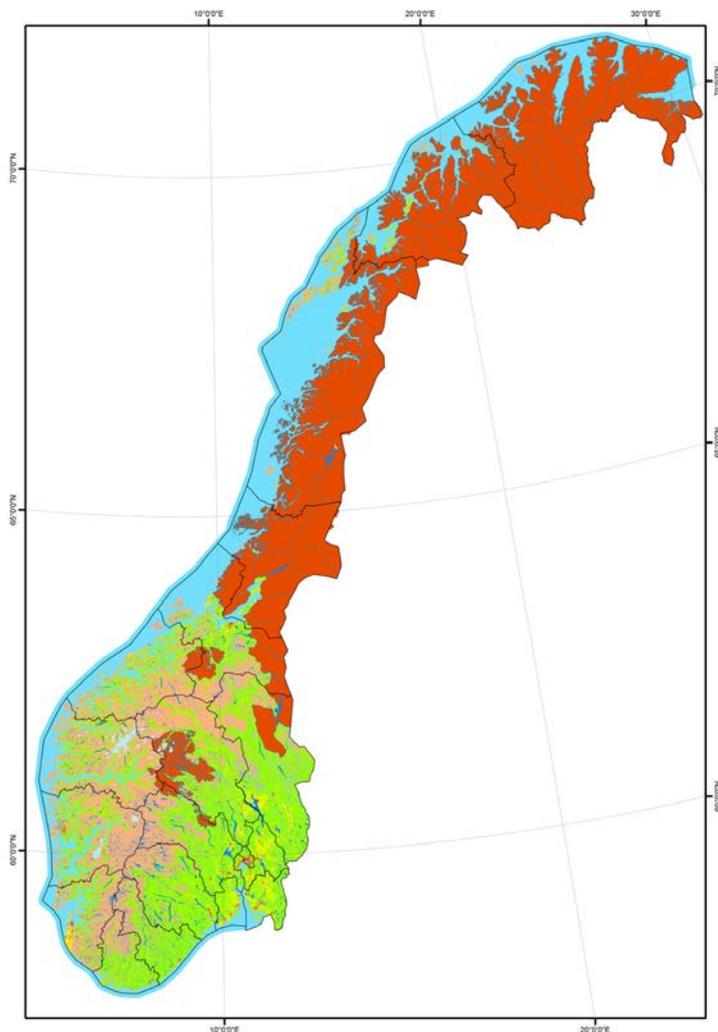


Figure 2-5. Reindeer herding (semi-domesticated reindeer; red coloured areas) is conducted in 140 municipalities in Norway, using around 140 000 km² or about 40 % of the land area of Norway (Map: Bernt Johansen, NORUT).

For the reindeer herding year 2012-2013, 3112 people were registered with a siida unit, with 246 262 registered reindeer, 180 000 animals (73 %) being in Finnmark County, and approximately 13 000 animals in Troms, 14 000 in Nordland, 14 000 in Nord-Trøndelag, 13 000 in Sør-Trøndelag and Hedmark Counties, and 12 500 in the non-Sami herding units ("tamreinlagene"). Of these animals, 75 % were females, 6 % were males, and 18 % were calves. For the whole reindeer herding, 85 % of the females gave birth to a calf in 2013, but 44 % of the calves and 10 % of the adult animals were lost during the year due to predators, starvation, killed by car, train etc., diseases, or other known and unknown factors. In 2012-2013, 75 761 reindeer were slaughtered, constituting 1 742 000 kg of meat. The mean slaughter weight was 18.8 kg (calf), 29.1 kg (female > 2 year) and 41.9 kg (male > 2 year). Due to differences in natural pasture resources, animal density, loss of animals to predators etc., the annual production of meat per female animal in the herd varied between districts, from almost nothing to up to 18.6 kg.

The reindeer herding units are designated to specific summer and winter pastures. A reindeer herder may share winter pastures with 5-10 other herds, and summer pastures with other herds. In a recent questionnaire about the disease keratoconjunctivitis in reindeer (63 respondents), almost 30 % of the responding herds reported contact with as many as 25 other herds or more during a year, sharing pastures, corrals, transport vehicles etc. (Tryland et al., 2016).

Although reindeer herding is organised in siidas, animals in most districts are free-ranging and without daily inspection. They can also be left without observation for longer periods to avoid disturbances, such as during calving. Although fences are sometimes used to limit mingling between herds, these are uncommon. Thus, reindeer of different herds will be in contact and to some extent intermingle. It is also common to exchange animals across siida borders and also over longer distances by road transport. Furthermore, reindeer owners often have to transport animals for slaughter for longer distances, and animals for slaughter may be sent to a distant slaughterhouse due to economic or other reasons. Thus, there will be contact between reindeer of different herds, which will include neighbouring herds, but also geographically more distant herds.

2.2.2 Moose

Moose (*Alces alces*) is further divided into several subspecies. *Alces alces* is usually called moose in American English, but sometimes referred to as "elk" in British English, whereas elk in North American English refers to wapiti deer (*Cervus canadensis*). In Europe they are therefore sometimes called Eurasian moose or Eurasian elk/European elk (*Alces alces alces*), which is mostly distributed in Finland, Sweden, Norway, Latvia, Estonia and Russia. In Norway, moose were previously restricted to the southern and eastern parts of the country, but in recent decades have spread to most parts of the country (Figure 2-6).

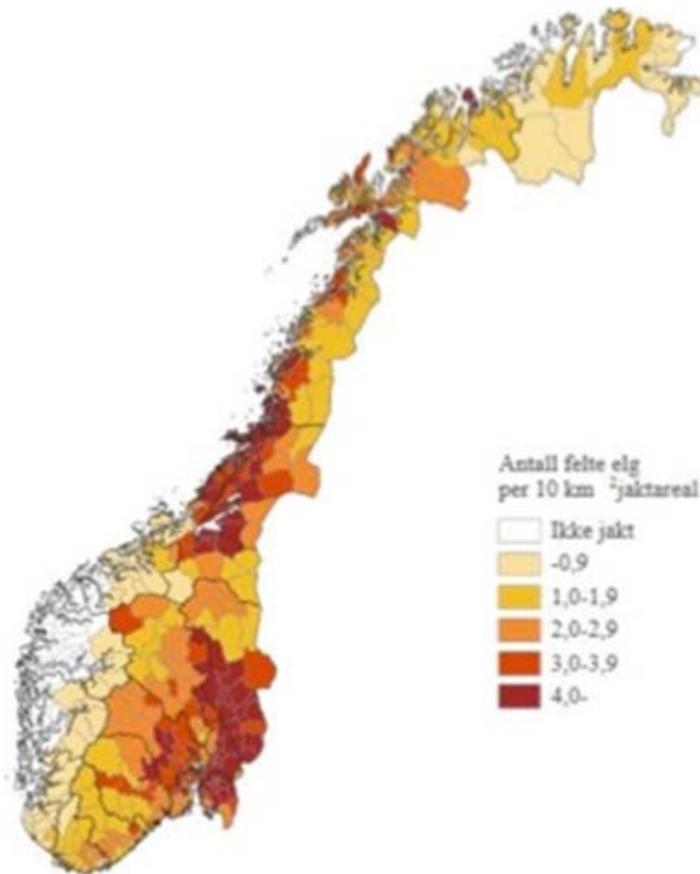


Figure 2-6. Number of moose shot per 10 km² hunting area (2013), reflecting the distribution of moose in Norway (Hjorteviltregisteret.no).

The moose is the largest cervid species, with males weighing 400-550 kg, females weighing 320-400 kg, and they may survive up to 15-25 years. A moose is brown to grey in colour and only males have antlers. They are commonly found in boreal coniferous and deciduous forests in temperate and subarctic regions on the Northern hemisphere. However, they may also exist in many other habitats, including regions with a mountainous and alpine character. Moose are solitary animals, with the strongest bonds between mother and calves, but dozens of animals may be observed together, especially during winter, when feed has been provided or under heavy snow conditions, to save energy. The total winter population of European moose in Norway is about 120 000 animals.

Males are polygamous, serving several females during the mating season. Moose usually give birth to one or two calves in May-June. The most common predators of moose in Fennoscandia are the wolf (*Canis lupus lupus*) and the brown bear (*Ursus arctos*). Moose hunting is a very popular activity, with long traditions in many regions, and is the main regulator of the populations, as predator numbers are low. The harvest of moose in Norway has been stable over the last decade, and approximately 32 000 animals were shot annually during 2005 - 2015 (www.hjorteviltregisteret.no).

Moose are selective feeders. In spring, they typically feed on fresh shoots of deciduous trees and shrubs and herbs, but also fresh grass. During summer, a full-grown moose may eat as much as 50 kg biomass each day, whereas during winter the metabolism is lower and the animal saves energy, and may eat only 8-16 kg biomass per day. The feed in winter is also less nutritious, consisting of older branches, also including pine trees. During winter, an adult moose may lose 20-25 % of its body weight (www.hjorteviltregisteret.no).

As described for reindeer, the winter and restricted food resources represent a major challenge for moose and the other cervid species. They may cope with heavy snowfalls and also severe cold, but a combination represents a major challenge, especially when the snow consists of ice layers. If the ice crust is thick enough to bear the weight of a moose, the animal will also have problems digging through the ice to find food. If the crust does not carry the animal, it will spend large amounts of energy seeking food, and the hair coat on the legs and the sides of the animal may be worn out. This sign is often found, along with emaciation (lack of fat resources -subcutaneous, intra-abdominal, coronary grooves and bone marrow) in moribund animals.

Various parasites are associated with moose, including lungworms and gastro-intestinal nematodes and protozoa. Moose can also harbour the brainworm, *Elaphostrongylus alces*, which can cause CNS symptoms, such as ataxia (Handeland and Gibbons, 2001). There are also reports of papillomatosis (papillomavirus) (Sundberg et al., 1985) and malignant catarrhal fever (gammaherpes virus) (Vikoren et al., 2006). Moose, along with other cervids, may be heavily infested by the blood sucking deer ked (*Lipotena cervi*), which is increasing in distribution in Norway, and is associated with hair loss (Madslien et al., 2011).

2.2.2.1 Moose wasting syndrome (MWS) in Sweden.

Since 1985 a wasting syndrome affecting moose (*Alces alces*) has been observed throughout Sweden, with an elevated occurrence in the county of Älvsborg, located in southwest Sweden. More than 1000 moose were found dead or euthanised in this County by the end of 1992 (Merza et al., 1994). This syndrome, designated moose wasting syndrome (MWS), is characterised by starved and emaciated animals displaying abnormal behaviour, including lack of normal flight behaviour, ataxia, lack of coordination, and circling movements. The aetiology of MWS is unclear (Frank, 2004) and investigations of brain material from affected animals for histopathological alterations revealed no spongiform changes characteristic of prion disease. Furthermore, immunohistochemical analyses for prion protein aggregates were also negative. Brain areas investigated included forebrain, cerebellum, and, importantly, the brain stem at the level of the obex. Based upon this, it was concluded that MWS was not caused by a prion agent like CWD (Rehbinder et al., 1991). In an investigation involving 25 field cases of MWS, pathological changes including erosions, ulcers, oedema and haemorrhages were found in the oral, oesophageal, and ruminal mucosa in all affected animals (Rehbinder, 2004). Despite the pathological similarities with those characteristic for Bovine Virus Diarrhoea /Mucosal Disease, this was not supported by histopathology. Furthermore, no pestivirus antigens could be detected in tissues and pestivirus could not be

isolated in cell cultures, but antibodies against pestivirus were detected in nine of 27 animals tested (virus neutralisation test) with highest titres against a roe deer isolate as compared with two cattle isolates (Rehbinder et al., 2004). A retrovirus previously isolated from diseased moose was suggested to be the cause of MWS (Merza et al., 1994), but this has gained little support as the only cause of the syndrome. Other hypotheses regarding the aetiology have been numerous, including, among others, several types of nutritional deficiencies (Frank, 1998).

2.2.3 Red deer

Red deer (*Cervus elaphus*) are distributed in most parts of Europe, the Asian part of Turkey, Middle-East, Asia, and North Africa. The coat of red deer is short and brown in summer, but in winter, the guard hairs become longer and greyer. A fully grown male can have a body length of 260 cm, a wither height of 150 cm, and weigh up to 250 kg, whereas a female typically weighs around 120 kg. Red deer can reach ages above 20 years, but rarely survive more than 10 years in the wild. Only males grow antlers. They are shed in April – May, and the development of new antlers starts immediately. In summer, they mainly feed on grass and sedges, and in winter they eat grass if available, but also heather and other plants, as well as shoots and branches from deciduous trees. Red deer females give birth to one calf (7 – 10 kg) in May, after eight months of pregnancy, and typically suckle the calf for about seven months.

2.2.3.1 Wild red deer

Red deer can be solitary or form groups, but the groups are considerably smaller than reindeer herds. Some individuals stay in the same area all year around, whereas others migrate between summer and winter pastures, typically from lowlands and forest areas on the coast to mountainous areas in the summer.

In Norway, red deer were originally distributed in coastal areas from south-western Norway (Rogaland County) to the border against Nordland County in the north, but during recent decades the distribution has increased, especially to the southern (Sørlandet) and eastern (Østlandet) parts of the country, and also northwards, reaching Nordland County. Figure 2-7 shows the harvest of red deer as number of shot animals per km². The harvest of red deer has been stable over the past years and, on average, 33 731 animals were shot annually in Norway during 2011 - 2015 (www.hjorteviltregisteret.no).

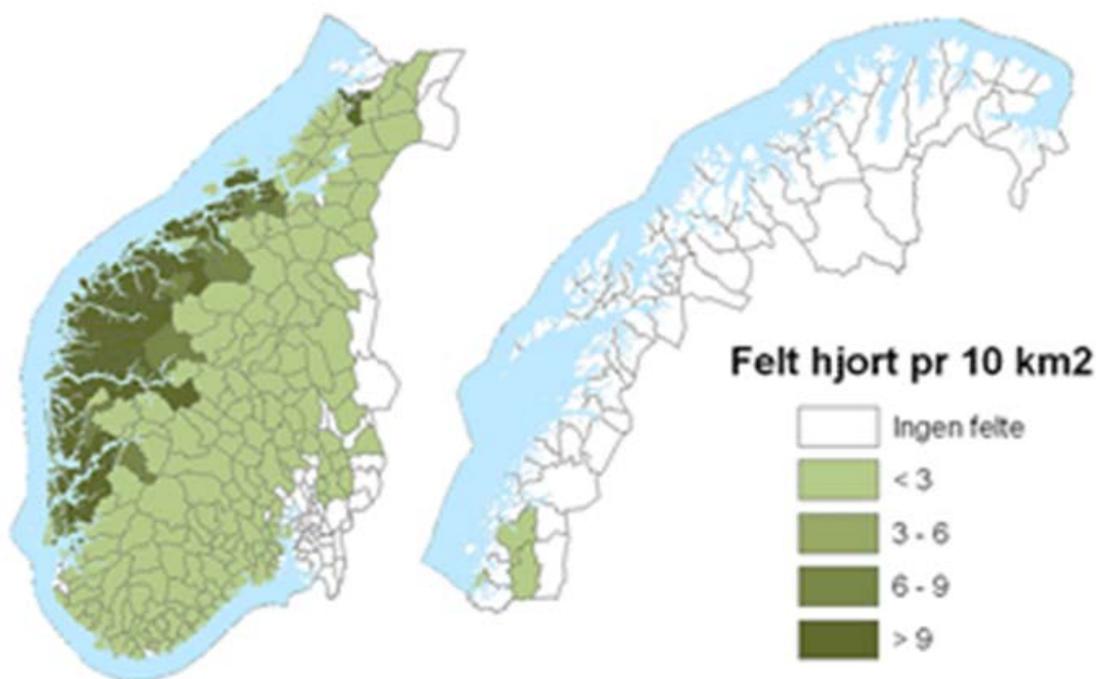


Figure 2-7. The harvest of red deer, *Cervus elaphus* (number of shot animals per km²), also reflecting the distribution of deer in Norway.

Gastro-intestinal parasites of deer have been included as part of the cervid surveillance programme (Hjorteviltovervåkingsprogrammet, HOP; unpublished data: (Vikøren et al., 2013)). As with other cervids in Norway, symptoms of malignant catarrhal fever and presence of gammaherpes virus has been verified in red deer in Norway. CNS symptoms from red deer include animals showing abnormal behaviour, such as apathy, incoordination, circling, staggered gait, convulsions, and impaired vision. Antibodies against gammaherpes virus were found in 13 animals (5 %) showing these symptoms (Vikøren et al., 2006). As for reindeer and moose, red deer may be infected by the brainworm, but with a species that is specific for red deer, *Elaphostrongylus cervi*. Upon experimental infection in red deer, this nematode caused encephalomyelitis, focal encephalomalacia and gliosis, meningitis, perineuritis, and other pathological changes in CNS (Handeland et al., 2000).

2.2.3.2 Farmed red deer

In Norway, deer farming started during the 1980s, and today, approximately 80 deer farms are in production, distributed mostly in the southern part of Norway, north to Fauske in Nordland County, and include about 8 400 animals (2014; <http://www.hjortesenteret.no>). About 150-200 tonnes of meat are produced per year (Pers. comm. Morten Nystad, Norsk Hjorteavlforening). Most farms have red deer, but some also keep fallow deer, or both red deer and fallow deer. Farmed deer are kept in enclosures and normally do not have contact

with free ranging cervids, but during the mating period, wild males may be able to enter enclosures to obtain access to females. In such cases, they are usually kept in the farm. In some instances, captive deer may escape the enclosures, which is how the wild population of fallow deer was established in Norway. Deer are usually shot inside the fence, bled, and transported to special facilities for deer that do not normally slaughter farm animals. Absence of tuberculosis and a general favourable health condition are the background requirements for free trade between deer farms. In addition to meat production, trade of livestock is an income for some farms, selling female calves of the year (six months old) or, more commonly, pregnant females (1.5 years old).

2.2.4 Roe deer

Roe deer is the smallest deer species in Norway, weighing 18-36 kg, with a wither height of 70-85 cm. The antlers (males) are shed in November-December, and new velvet-covered antlers (no: bast) soon established. The males use small trees to clean the velvet from new antlers (April-May) and to mark territory, until after the rut in August. Roe deer were almost extinct in Scandinavia around 1830, with only a small population left in Skåne, Sweden, which was protected. They are now abundant in South-Eastern Norway (Østlandet), but after 1930-1940 its distribution expanded to most parts of Norway, and has also been established in Finnmark County from 1980. The Norwegian population is estimated to consist of approximately 150 000 animals.

Roe deer is found in agricultural land and grasslands, but also different types of forests. They live solitary or in small family groups, but may also gather in groups of a few dozen, depending on food availability. They eat grass, rushes and sedges, and berries, but also grain, heather, and leaves.

The harvest of roe deer has in general been stable over the past decade, but with a peak of 30 790 animals for the season 2009 – 2010. On average, 26 732 roe deer were hunted annually during 2011 – 2015 (Statistics Norway; www.ssb.no)

Roe deer is an important host for ticks (*Ixodes ricinus*) in many ecosystems, and antibodies against *Anaplasma* have been detected in roe deer (Tveten, 2014). The disease malignant catarrhal fever, caused by gammaherpesvirus, has been detected in a few roe deer (Vikøren et al., 2013). Antibodies against alphaherpesvirus and pestivirus have been detected in roe deer from southern Norway and Nordland County, in 3.0 % (n=602) and 12.3 % (n=635), respectively, suggesting that pestivirus is enzootic in the roe deer population (Lillehaug et al., 2003). There are no records of conditions that affect the CNS of roe deer in Norway.

2.2.5 Fallow deer

Wild fallow deer in Norway originate from individuals that have escaped from deer farms. A small population has settled on Hankø Island (Østfold County) and individuals are sometimes observed east of the river Glomma (Hedmark County). With its limited distribution and

separation from reindeer populations, fallow deer are presently regarded as having little relevance to the CWD case in reindeer in Nordfjella. However, given the shared geographical distribution with other cervids, such as moose, roe deer, and red deer, and the new CWD cases in moose, fallow deer should also be considered when addressing the epidemiology of CWD in Norway.

Fallow deer have characteristic white spots on the sides and over the back. Only males have antlers, which are shed in April-May. Fallow deer is a small deer species, smaller than red deer, but larger than the roe deer. An adult female fallow deer (> 3 years) weighs about 40-65 kg and an adult male (> 5 years) weighs approximately 80-120 kg. After approximately eight months pregnancy, one calf is born. Fallow deer are not herd animals like reindeer, but usually appear in larger groups than red deer. Females and calves typically form their own groups in other periods of the year than the rut, which starts in October-November, when males may fight and gather a harem of females.

Fallow deer are often seen on open agricultural land, preferring grass pastures in the spring and summer, but usually return to the forests during autumn and winter to seek more shelter, and to feed on nuts, berries, and bark. They are regarded as stationary compared with other deer species.

2.2.6 Harvesting of cervids in Norway

Characteristics of the populations of cervids in Norway and the numbers of slaughtered (semi-domesticated reindeer) and hunted cervids are outlined above (see 2.2).

Hunting statistics show that in the Nordfjella region and adjacent municipalities, a total of 268 moose and 679 red deer were shot (2015). Of these, 41 % and 48 % of the moose and red deer, respectively, were classified as "old", as compared with adults and calves.

Table 1. Harvesting of moose and red deer from the municipalities including and surrounding Nordfjella in 2015. (www.hjorteviltregisteret.no)

Municipality	Species	Year	Area, km ²	Calves	Adult	Old	Total
Hemsedal	Moose	2015	279.9	16	16	23	55
Ål	Moose	2015	454.0	35	37	53	125
Hol	Moose	2015	426.7	25	25	27	77
Ulvik	Moose	2015	124.3	1	0	0	1
Aurland	Moose	2015	297.4	0	1	4	5
Lærdal	Moose	2015	233.5	1	0	4	5
Hemsedal	Red deer	2015	279.9	6	7	16	29
Ål	Red deer	2015	499.5	3	5	13	21
Hol	Red deer	2015	426.7	6	10	16	32
Ulvik	Red deer	2015	114.0	14	27	38	79
Aurland	Red deer	2015	322.5	38	49	76	163
Lærdal	Red deer	2015	364.3	89	97	169	355

Selbu municipality, where the two moose with CWD were found, consists of 1147 km² land. Based on hunting statistics (www.hjorteviltregisteret.no), moose were not hunted in Selbu for a three year period (2006 – 2008), whereas an average of 336 animals have been shot annually for the past five years (2011-2015), indicating a stable moose population for this period. For roe deer, no animals were registered as hunted prior to 2011, whereas for the period 2011 – 2015, 212 animals were shot, with an average of 42 roe deer per year. For red deer, no animals were registered shot prior to 2009. For the period 2011 – 2015, 183 red deer were reported harvested, increasing from 22 in 2011 to 46 in 2015 (average 36 animals per year), indicating an increasing population. These data suggest that both roe deer and red deer are relatively new species in this region, with increasing populations.

2.2.7 Shared habitats and contact between different cervid species in Norway

Cervid populations in Norway have generally increased during recent decades, and, including the semi-domesticated reindeer herds, all cervid species (except the fallow deer) in Norway may be found in most parts of the country.

The different cervid species have somewhat differing dietary preferences and these may change during the seasons. Searching for food, together with seeking shelter and security, are important drivers of animal movement and seasonal migrations. Free-ranging cervids generally have a restricted energy uptake during winter, and in the spring seek pastures and regions with an early onset of greening, such as in coastal and low land regions. It is thus common to see cervids of different types feeding on farmland pastures that are free of snow early in the spring. In summer, domestic animals, such as sheep and goats, increasingly graze high altitude and mountainous pastures, thus increasing the potential for contact with wild cervids. Some free-ranging cervids, such as the moose, may also visit farmland during winter and feed upon silage bales. In fact, local moose might become a problem for the farmer, as they open the bales, letting in air in and destroying the feed.

Feeding wild cervids, such as roe deer and red deer, is rather common in Norway. The purpose of this may be to keep animals away from traffic points and busy roads, but also to increase the number of animals that survive the winter. If winter feed is made available in a sheltered spot, moose, red deer, and roe deer may have greater contact than would normally occur with natural feeding. Such feeding areas should be considered as potential environmental hot-spots that could facilitate direct and indirect transmission of infectious agents, including prions.

2.3 Prion diseases in Norway, surveillance and occurrence

2.3.1 Surveillance and occurrence

Scrapie has been a notifiable disease in Norway since 1965. The decision for this classification was the information from other countries about the serious character of this disease, but also because sheep scrapie had been diagnosed in 1958 in two rams in an isolation facility in Norway. Initially, surveillance was passive and based on clinical observations by veterinary practitioners, with support of diagnostic competence and capacity in the research institutions, the Norwegian Veterinary Institute and the Norwegian School of Veterinary Science (now a faculty at the Norwegian University of Life Sciences, NMBU). In 1981, the first case of scrapie in indigenous sheep was diagnosed. A series of single flock outbreaks occurred during the 1980s and early 1990s, and scrapie was diagnosed in eight flocks in 1995 and 31 flocks in 1996. In 1997, an active surveillance programme of slaughtered animals was introduced. All farmers were given information about the disease, including the requirement to report cases with typical symptoms to the district veterinary officer.

A diseased sheep with clinical signs unusual for classical scrapie was detected in 1998. This animal carried a PrP genotype that was rarely seen in classical scrapie and, interestingly, flocks-mates aged 5-6 years with PrP genotypes considered highly susceptible were observed (Tranulis et al., 1999). Based on differences in clinical symptoms, distribution of prion protein in the brain, molecular profile on western blot, and genotype a new type of scrapie was described, named Nor98 scrapie, later atypical scrapie (Benestad et al., 2003).

In Norway, both classical and atypical/Nor98 scrapie were treated with stringent measures, destroying all animals in the affected flock as well as contact flocks. As a result of this, more than 70 000 sheep were destroyed, based solely on contact with diagnosed scrapie cases. Further epidemiological (Fediaevsky et al., 2008; Hopp et al., 2006), genetic (Moum et al., 2005), and pathological studies from Norway and many European laboratories revealed important differences between classical and atypical/Nor98, which subsequently led to alterations in Governmental regulations regarding these diseases. Importantly, whereas classical scrapie was clearly infectious, data concerning atypical/Nor98 scrapie suggested that this disease was far less contagious, with normally only single cases observed in flocks. Sporadic occurrence of atypical/Nor98 scrapie is possible.

The UK BSE outbreak (1986-1998) was followed with close interest in Norway. Histology of brain tissues was included in necropsy of suspected animals. In 1994, a case of feline spongiform encephalopathy was diagnosed in a cat that had been fed feed imported from England (Bratberg et al., 1995).

Verification of BSE as a zoonotic disease resulted in instigation of surveillance programmes in accordance with European Commission Regulations. The BSE programmes in Norway were based on passive surveillance between 1998 and 2000, and active surveillance from May

2000 onwards. During the first couple of years, samples were investigated by histological examinations, and from 2001 an ELISA method was used. Approximately 270 000 samples have been analysed to date, all negative for classical BSE. However, in 2015 atypical BSE was diagnosed in a cow included in the surveillance programmes.

Table 2. Animal prion diseases diagnosed in Norway (1981 – June 2016).

Species	Classical scrapie*	Atypical/Nor98 scrapie	Atypical BSE (H-type)	FSE	CWD
Sheep	63	132			
Goat		1 (2005)			
Cattle			1 (2015)		
Domestic cat				1 (1994)	
Reindeer (wild)					1 (2016)
Moose					2 (2016)

*Prior to 1998, classical and atypical/Nor98 were not distinguished.

In 2015, atypical BSE (H-type) was diagnosed in a 15-year old cow of the Scottish Highland breed, which was killed due to injury. In 1994, a cat was diagnosed with feline spongiform encephalopathy (FSE) (Bratberg et al., 1995). A single case of atypical/Nor98 scrapie was diagnosed in a goat in 2005, and, in the following years, 3-15 cases of atypical/Nor98 scrapie in sheep were identified annually. Classical scrapie is rare in Norway now and only six single-flock outbreaks (in 2001, 2002, 2003, 2004, 2006 and 2009) have been diagnosed during the last 15 years (Table 2).

During the slaughter season of semi-domesticated reindeer in the municipalities of Kautokeino and Karasjok (Finnmark County) from December 2003 until February 2004, samples from 792 animals were collected (Sigurdur Sigurdarson, Keldur, Iceland and Morten Tryland, NVH, Norway) and screened for prion diseases (Bjørn Bratberg and Sylvie Benestad, NVI, Norway), and found negative (S. Sigurdarson, Keldur, Iceland, pers. communication).

During 2006-2010, a survey of European cervid populations for CWD was carried out. This involved 21 EU Member States and Norway, and approximately 13 000 brain stem samples were analysed – all with negative results (EFSA, 2010). This led to the conclusion that there is currently no CWD epidemic in European cervid populations. However, due to the limited sample size, low-level occurrence could not be excluded.

Of particular relevance to the Norwegian CWD cases, Table 3 summarizes TSE testing of cervids and muskox in Norway.

Table 3. Overview of Norwegian cervids and muskox tested for prion disease (NVI).

Species*	Roe deer	Red deer	Fallow deer	Reindeer (wild)	Reindeer (Semi-dom.)	Moose	Muskox	Sum
2004	21	11	0	1	792	10	13	848
2005	17	10	0	1	93	14	10	145
2006	9	129	0	0	48	12	13	211
2007	34	612	8	0	30	35	1	720
2008	26	9	0	2	0	9	1	47
2009	31	9	0	2	0	11	0	53
2010	17	5	0	2	0	13	4	41
2011	12	13	0	1	1	11	0	38
2012	3	10	3	0	0	5	0	21
2013	4	4	1	0	0	1	0	10
2014	1	4	0	0	0	5	0	10
2015	8	4	0	3	0	4	0	19
Total	183	820	12	10	966	130	42	2163

*Roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), reindeer (*Rangifer tarandus tarandus*), moose (*Alces alces*), muskox (*Ovibos moschatus*).

Of note, by May 2016, 34 wild reindeer had been tested by NVI, all with negative results except the single CWD case from Nordfjella. Of the tested animals, 14 were from the Nordfjella population, however, only three were adults, the others being calves. As all the semi-domesticated reindeer tested (n=792, 2004) were from Finnmark County, and few animals of other species have been tested, the data are insufficient to draw any conclusions regarding CWD prevalence.

The surveillance programmes described above have been of pivotal importance for several areas of scrapie research in Norway. The awareness and dedication of all stakeholders in Norwegian livestock, reindeer herding, and wildlife management participating in surveillance of animal prion disease in Norway cannot be overemphasised. For more details on research see Appendix II.

3 Exposure assessment

For the purpose of this opinion, the following terminology related to assessment of exposure and risk will be applied (OIE, 2004):

- Negligible: So rare that it does not merit consideration
- Very low: Very rare, but cannot be excluded
- Low: Rare, but does occur
- Medium: Occurs regularly

- High: Occurs often
- Very high: Event almost certainly occurs

3.1 Exposures leading to introduction to Norway

A detailed assessment of the probable route of introduction of CWD to Norway is currently not possible and therefore potential routes of introduction are discussed in general terms.

3.1.1 From North America.

As CWD had not been detected in Europe previously, introduction from North America is considered the most likely route of introduction. Possible routes of introduction to UK from North America have recently been discussed in an updated qualitative risk assessment published by the UK Department for Environment Food & Rural Affairs (DEFRA, 2016).

The assessment and conclusions presented in the DEFRA report are considered relevant for Norway. If CWD was imported into Norway from North America, we consider that the potential routes of introduction are:

- Import of deer or moose urine lures for hunting purposes
- Import of CWD prion-contaminated equipment and/or clothing/footwear of hunters or other tourists
- Import via non-ruminant feed or pet food is considered unlikely

3.1.2 From other European countries

Norway shares borders with Sweden, Finland, and Russia. Animals migrate, and have been transported, to and from Sweden and (in the north) Finland in particular. Although not documented, there is a chance that CWD has been hiding (below the level of detection) in the joint Fenno-Scandinavian cervid populations for years, and introduction to Norway might have occurred from neighbouring countries.

3.1.3 Spontaneous occurrence of CWD

Growing epidemiological and experimental evidence suggests that certain forms of ruminant prion disease can occur in a manner similar to sporadic Creutzfeldt-Jakob Disease in humans, with a spontaneous occurrence at a steady, but very low, level. This might be the case for atypical/Nor98 scrapie in sheep and atypical forms of BSE affecting cattle, and cannot be excluded for CWD. A low level occurrence of CWD in wild cervids would probably go unnoticed, but it is less likely that a low level of occurrence of CWD would go unnoticed in the herds of semi-domesticated reindeer.

3.2 Exposure of wild and semi-domesticated reindeer populations

Global positioning system (GPS) tagging of wild reindeer in Nordfjella Mountains (2008 – 2010) showed that very few animals used the south and east regions of the area at that time, and that most animals were gathered, for all seasons combined, in the western parts, bordering the municipalities of Aurland and Lærdal (Figure 3-1).

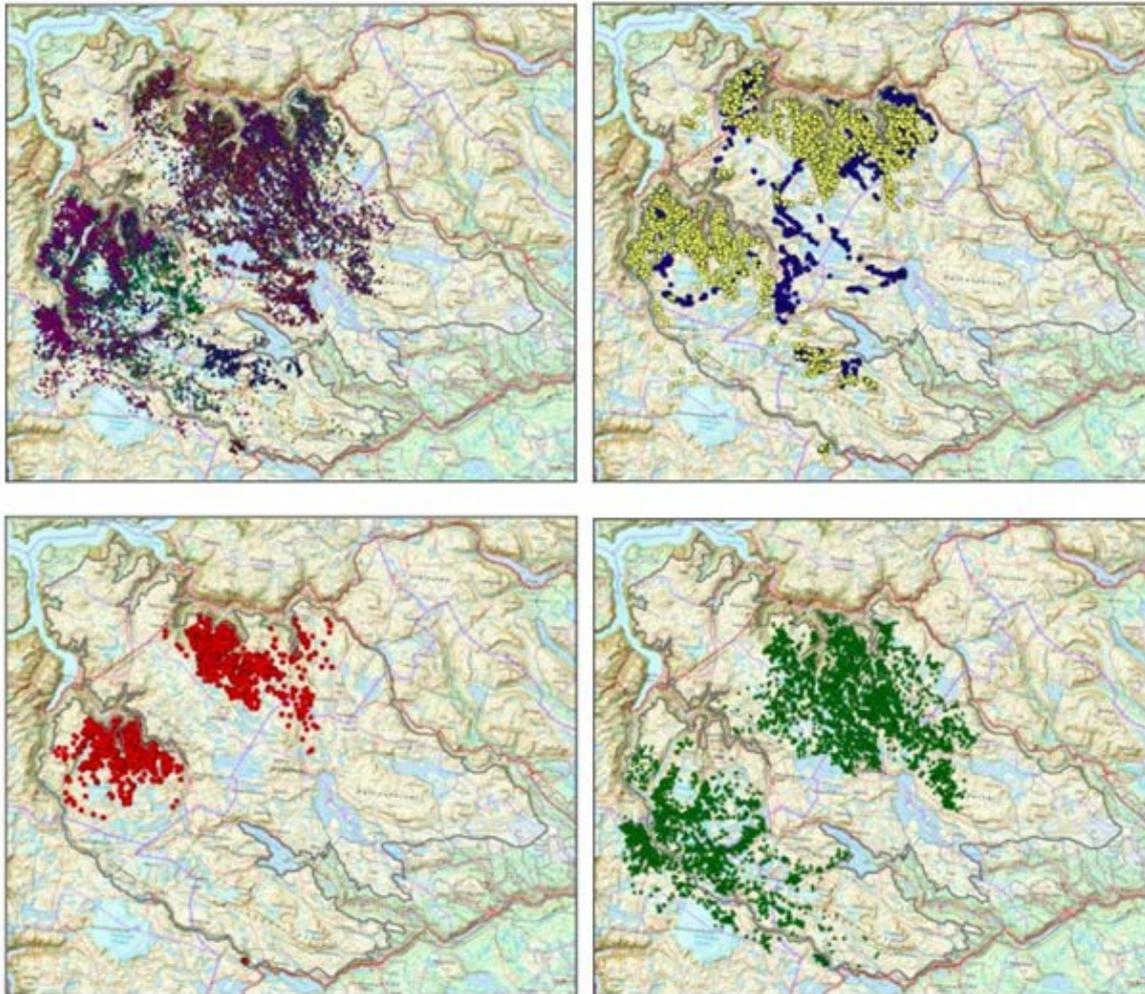


Figure 3-1. GPS data showing the spatial use of the Nordfjella pastures (2008 – 2010) by the wild reindeer population: upper left, all periods combined; upper right, during winter (blue = January and February, yellow (March and April); lower left, during May (calving); and lower right, during summer (July and August) (Strand et al., 2011).

Since 2006, wild reindeer have migrated from the Hardangervidda mountain plateau in the south into Nordfjella. Similarly, each year, approximately 1000 reindeer from southern parts of Nordfjella (Forvaltningsområde II – Management zone II) have used summer pastures south of the Bergensbanen railway, and a herd of approximately 100 animals have used pastures west of Hardangerjøkulen glacier during the winter (Strand et al., 2011).

The Nordfjella wild reindeer management region (Figure 2-4, shown in red) currently borders the Hemsedalsfjella mountain region north of the road Riksveg 52, which is a part of the non-Sami reindeer herding (Figure 2-4, shown in pink). There is still some contact between the Nordfjella wild reindeer and the semi-domesticated reindeer in the north, the latter sometimes entering the Nordfjella management zones (Strand et al., 2011).

The herd structure and seasonal migration pattern of the wild reindeer population in Nordfjella Mountains allow contact with other reindeer populations, both wild reindeer and semi-domesticated, and transmission of CWD between these populations could be possible. There are insufficient data to estimate the probability of exposure of wild and semi-domesticated reindeer in the areas surrounding Nordfjella Mountains to the CWD agent originating from the affected population.

3.3 Exposure of moose, red deer, and roe deer

On the basis of current knowledge of CWD, all cervid species found in Norway should be considered potentially susceptible to CWD.

In the Nordfjella Mountains, moose, red deer, and roe deer show partial habitat overlap with reindeer. Although their primary habitats are in the coniferous and deciduous forests surrounding Nordfjella, reindeer are also regularly encountered in the sub-alpine and low-alpine region. Likewise, reindeer may graze in sub-alpine forest during summer. There are insufficient data to estimate the probability of exposure in the reindeer population.

Selbu Municipality consists of 1147 km² land. The region where the two diseased moose were found is frequented by moose, roe deer, and red deer, with moose and roe deer being the most abundant species. These species are sympatric, existing in the same ecosystems and feeding partly on the same plants. Whereas the moose population seems to be stable, based on hunting statistics the red deer and roe deer populations seem to be recently established and increasing (see 2.2.6). The moose population is regarded as the most important species in this region in terms of transmission of prions, both within the moose population and also to other species. It is also assumed that the moose, red deer, and roe deer populations are shared with Sweden (Jämtland). There are insufficient data to estimate the probability of exposure among moose and other cervids in that area.

The moose population in Selbu may have significant contact with semi-domesticated reindeer in this region. There are insufficient data to estimate the probability of exposure of wild and semi-domesticated reindeer in the area.

3.4 Exposure of sheep, cattle, and goats

Sheep, cattle, and goats may be exposed to CWD via grazing areas shared with cervids. The probability of exposure cannot be estimated at this time, due to a lack of data on the

prevalence of CWD among cervids. Sheep and cattle have been shown to be poorly susceptible to CWD (Sigurdson, 2008). Hence, sheep and cattle are at very low risk of developing CWD, and transmission of CWD to grazing sheep or cattle seems unlikely (Hamir et al., 2011; Hamir et al., 2005; Hamir et al., 2007) (see 2.1.9.1).

3.5 Exposure of humans

In the present situation, there are insufficient data to estimate the probability of direct and indirect exposure of humans to CWD prions.

In North America, human exposure to the agent via consumption of venison in the enzootic areas is well documented (Sigurdson, 2008). The main risk of exposure to material containing prions from CWD will, however, be through contact with hunted or slaughtered animals.

One particularly well-documented case of point-source exposure to CWD-contaminated venison has been described from Upstate New York, in which more than 200 participants at a Sportsman's barbecue party were exposed to CWD (Olszowy et al., 2014). In response to this, The Oneida County CWD Surveillance Project was launched that will provide a unique opportunity for long-term follow-up of exposed individuals.

Although, data accrued to date provide no evidence of CWD causing disease in humans, implementation of precautionary measures to reduce exposure is recommended.

4 Risk characterisation

Based upon the preceding text, the following paragraphs summarise the main factors that determine the possible scenarios in Norway in the present situation of detection of CWD in one wild reindeer and two moose.

4.1 Prions

- Prions are among the most resilient pathogens known. Any dissemination of prion agents into ecosystems constitutes a potential long-term problem. If CWD prions are disseminated to the environment from a diseased reindeer or moose with a similar efficiency to that previously observed in other species of deer, infective prion particles will persist in the environment, particularly at sites where infected carcasses have decayed. However, the probability of infectivity being transferred between animals via direct contact is likely to be much higher than via abiotic or biotic reservoirs.
- The CWD agent can bind to soil particles, and plants can take up CWD prions from soil and convey them to other parts of the plant, including leaves. Hence, grass and other plants may have the potential to transmit CWD to grazing cervids.

- In CWD generally, and in experimental CWD in reindeer, most, if not all, peripheral organs must be considered as being contaminated with prions, and consequently infectious.
- Clinical signs of CWD are non-specific and diagnosis based solely on symptoms is not possible.
- Diagnosis is determined by examination of samples from the medulla oblongata at the level of the obex. Samples from peripheral organs are insufficient for diagnostic or surveillance purposes.
- Bioassays are needed for direct estimate of infectivity. PMCA provides a highly sensitive method for quantifying misfolding/converting activity and this provides a good approximation of prion infectivity.

4.2 Populations

- Horizontal transmission (direct and indirect) of CWD is known to occur and migration of animals (wild and semi-domesticated) is relevant for the spread of the disease between areas. However, sporadic or genetic (somatic mutation) prion disease development in cervids cannot be completely excluded.
- Although data are limited, it is likely that PrP genetic variation in reindeer confers varying susceptibilities towards CWD infection and that the S -> N substitution at codon 138 reduces susceptibility. Mapping of PrP genetic variation in wild and semi-domesticated reindeer, as well as in other cervid species, in Norway is recommended.
- All non-experimental prion diseases identified in a cervid species should, by definition, be named CWD. Thus, there is no doubt that the diseased reindeer and moose suffered from CWD. However, this alone provides little information on the characteristics of the prion agent isolated from the three cases diagnosed in Norway and described in this report.

4.3 Exposure

- The origin of CWD and its geographical distribution in Norway is currently unknown.
- Domestic ruminants like cattle, sheep, and goats might be exposed to CWD on pasture, either via direct contact with cervids or indirectly. Due to low susceptibility, the probability that cattle, sheep, and goats develop CWD, even following exposure, is considered very low.
- While humans may be exposed to CWD prions through hunting, slaughtering, or other direct or indirect contact with cervids, including consumption of cervid meat, there are no documented cases of zoonotic transfer of CWD to humans, even in regions where CWD is enzootic. Thus, the probability of human disease caused by CWD is considered very low.

4.4 Summary of risk characterisation

Table 4. Estimated probability of exposure and development of CWD after exposure, Norway, as for June 2016

	Probability ¹	
	Exposure ²	CWD development post-exposure
Cervids		High
- Nordfjella Mountains	Insufficient data	
- Selbu Municipality	Insufficient data	
Sheep, cattle, goats		Very low
- Nordfjella Mountains	Insufficient data	
- Selbu Municipality	Insufficient data	
Humans		Very low
- Hunting	Insufficient data	
- Meat	Insufficient data	
- Berries, water	Insufficient data	

¹ For the purpose of this opinion, the following terminology related to assessment of exposure and risk is used (OIE, 2004): see Exposure assessment.

² Due to lack of data, exposure probabilities cannot be estimated at this time.

5 Uncertainties and data gaps

At the present level of knowledge, there are several uncertainties and data gaps:

- The prevalence and spatial distribution of CWD in wild and semi-domesticated cervids in Norway are unknown. A national screening programme is warranted.
- The origin of introduction (if introduced) remains unknown.
- It is highly unlikely that the three cases detected so far, with a distance of 300 km between the first case and the other two cases, all emerged spontaneously, but the possibility of spontaneous occurrence in at least one of them cannot be excluded.
- Transmission properties, including zoonotic properties, of the Norwegian CWD isolates are unknown. Investigations are warranted and are ongoing.
- The genetic variation in PrP in wild and semi-domesticated cervids in Norway, and thus the susceptibility to CWD is unknown. Investigations are warranted.

6 Conclusions (with answers to the Terms of Reference)

Based upon the available evidence and current situation in the field, we present our conclusions starting with the animal disease situation, then the zoonotic risk. We refer to the terms of reference as given by the abbreviation "ToR". Thus "ToR 1.2" refers to the questions under 1.2 in the Terms of Reference chapter. It is important to note that all our conclusions are based upon the status of knowledge as of June 2016. Options for risk management are likely to change as more information becomes available, for example when results from the intensive surveillance are available from August 2016.

6.1 ToR 2

6.1.1 CWD in cervids

The origin of CWD in the three cases diagnosed in Norway remains unknown, and it is unknown whether the origin is the same for each case. The current situation may represent serious health and welfare problems for wild and semi-domestic cervids in Norway. In addition to the impacts on wild cervid populations, Sami reindeer herding as we know it today, will be severely challenged should CWD become enzootic in the reindeer herds.

We want to underline that this has to be treated as a possible emergency situation. Not only will our efforts now influence how our cervid populations will develop, but a lack of appropriate management may result in serious consequences for our neighbouring countries and European/Asian countries in general.

6.1.2 Transmission within and between cervids (ToR 2.1, 2.2, 2.3, 2.5, 2.7)

CWD spreads easily among cervids, with apparently little or no species barriers. If not controlled, there is a high risk that CWD will spread to cervids other than reindeer and moose, and potentially also to areas outside Norway.

As CWD transmits easily between cervid species it may spread over considerable distances through animal migration. Disease transmission will be influenced by flock structure (large flocks, as with reindeer, will facilitate transmission) and transmission rate will increase with increasing population densities.

Areas with very high population concentrations, such as around feeding places, sites for mineral licking stones, or whenever semi-domesticated reindeer are corralled, should be considered as hot-spots for disease transmission. Transport and/or movement of wild and semi-domesticated cervids in Norway may also include crossing the borders to Sweden,

Finland, and Russia. The agreement between Norway and Sweden regarding sharing of reindeer pastures on both sides of the border at certain times of the year might be re-considered in the present circumstances.

Data from North America suggest that anthropogenic factors are important for transmission, especially concerning spread over large distances. Such spread results from transportation of live cervids, carcasses or products thereof, and through the movement of people with contaminated footwear, clothing, or equipment (particularly hunting equipment).

CWD is transmitted horizontally through physical animal contact or via environmental sources. Infective prions may be found in faeces, urine, saliva, placenta and amniotic fluid, milk, and epidermal debris (skin rubbings). Shedding of prions in urine, saliva, and faeces occurs over prolonged periods. Repeated exposure to materials with low-level contamination may be of relevance. Prions in saliva are also important because very large volumes of saliva are swallowed daily. This has the potential to re-infect the gastro-intestinal tract and could increase faecal excretion.

Silage may contain the prion agent if harvested from areas frequented by cervids and also lead to elevated animal congregation. As prions can remain infective after more than two years in harsh environments, contaminated grass and silage cannot be ruled out as potential transmission vehicles.

The CWD agent can bind to soil particles, and plants can take up CWD prions from soil; in laboratory experiments disease has been transmitted via plant material. This indirect route is, however, considered to be of less relevance than direct exposure to infected animals.

6.1.3 Control of CWD in cervids in Norway (ToR 2.4, 2.6, 2.8)

Control and eradication of CWD in wild cervid populations is currently considered to be very difficult. If CWD has already disseminated into cervid populations in Norway, implementation of measures such as culling of old animals, reducing population densities, or physical separation of populations may be relevant. However, our lack of knowledge about the actual prevalence of CWD in cervid populations Norway argues against the implementation of strict control measures at the current time, as it would not be possible to introduce them on a rational basis. Similarly, implementing control measures that have already been tried in USA and Canada is premature in the absence of data on prevalence and other characteristics of CWD in Norwegian cervid populations.

The following surveillance and control measures are recommended based on our current level of knowledge and on the situation in the field as of today:

1. Surveillance

- a. Active helicopter surveys with trained staff should be conducted in the two hitherto affected regions (July 2016) and should be used to identify animals with clinical

symptoms resembling CWD. Such animals should be euthanised and necropsied with particular emphasis on identification of CWD.

- b. Heads of all hunted or slaughtered cervids (including semi-domesticated reindeer and farmed deer) above 2 years of age should be collected for sampling of the medulla oblongata.
- c. Any cervid with symptoms similar to CWD should be shot and reported to authorities. Increased numbers of wild cervids should be hunted in areas where CWD has been documented, with a focus on harvesting and sampling as many animals as possible that are two years or older. Regular hunting quotas should not include these animals. Such increased harvesting would result in decreasing the population density and this might be an additional advantage in terms of reducing the possibility of transmission.
- d. Authorities, reindeer herders, hunters, tourists, farmers, and the general public should be requested to report all dead cervids, as well as any cervids with abnormal behaviour, to the authorities. A public information campaign should be launched, targeting hunters, herders, farmers, and people engaged in other outdoor activities like hiking and camping, and people visiting holiday cabins to report relevant observations for follow up.
- e. Sampled "healthy" animals should not be embargoed; handling of carcasses from animals showing any symptoms or fallen stock should be the responsibility of the authorities. Only healthy animals should be intended for food.

2. Analysis of medulla oblongata samples

- a. Samples from animals showing symptoms associated with CWD or dead animals should be prioritised.
- b. Samples from healthy animals should be analysed by region in order to map regions of occurrence and apparent absence of occurrence.

3. Control of CWD in wild Norwegian cervids

- a. Feeding wild cervids should be prohibited by law to reduce congregation of animals and to reduce contact between cervid species. Farmers should be encouraged to try to restrict cervids from feeding on hay/silage bales left in fields. Farmers should be requested to remove old bales from their fields.
- b. Licking stones for cervids should not be provided and those in place should be removed. In CWD regions, areas previously used for licking stones for cervids should be fenced.
- c. Live cervids and cervid carcasses should not be transported across county borders without special permission from the authorities.

- d. Import and use of urine lures originating from cervids in enzootic areas should be banned.
- e. Hunters should not use equipment used in CWD areas for other hunting areas. Hunters and hikers returning from/coming from CWD areas in North America should be informed that their gear might be contaminated with infectious material that could transmit a fatal disease to Norwegian cervids. Information on how their equipment can be cleaned and washed should be provided.

4. Control of CWD in semi-domesticated reindeer

- a. Herders should be informed about the CWD situation and be kept updated on the possible implications for reindeer herding.
- b. Herding activities in and around affected areas should be mapped in order to clarify movements of animals (seasonal migration, slaughter etc.) and to identify contact herds.
- c. Reindeer herding across country borders (Sweden, Finland; "grenseoverskridende reindrif") should be mapped, and should be banned if associated with CWD-affected regions.
- d. Transfer of live animals from CWD-affected regions to other herds than those already identified as contact herds should be banned.
- e. Live reindeer and reindeer carcasses should not be transported across county borders without special permission from the authorities.

5. Crossing the species barrier to other animals

CWD can easily spread and cause disease within and between cervid species, but is not likely to spread to or cause disease in domestic ruminants or other animals. There is no indication that CWD in Norway stems from scrapie in sheep or BSE in cattle.

6. Surveillance and control in domestic ruminants

We consider the current surveillance programme for cattle, sheep, and goats to be sufficient as a system to detect transmission from cervids. As licking stones for domestic ruminants may also lead to congregation of cervids, removal of licking stones for domestic ruminants from areas where CWD has been documented should also be considered.

6.2 CWD as a possible zoonosis and risk for human health (ToR 1)

6.2.1 Zoonotic potential (ToR 1.1)

There are currently no epidemiological data that link CWD to human prion disease.

Although transmission of CWD to humans has never been known to occur, and data from bioassays and *in vitro* experiments indicate a species barrier, this possibility cannot be excluded.

Taking into account uncertainties regarding the plasticity of the CWD agents and the lack of transmission data from the Norwegian isolates, this scientific opinion considers the zoonotic risk of CWD to be very low.

Human health risks must be continuously assessed through results from surveillance and characterisation of prion isolates. Any study or research that indicates the possibility of zoonotic transfer must be taken into consideration.

At our current stage of knowledge, we recommend that the principal focus is directed towards collecting as much information as possible about the occurrence of CWD in Norwegian cervid populations, both wild and semi-domesticated.

6.2.2 Direct or indirect exposure from animals (ToR 1.2, 1.3)

- a. Since any dead cervids or any animal showing symptoms indicative of CWD should be handled by the authorities and their trained staff, the risk to hunters or slaughterhouse staff should be negligible. However, the information must be circulated widely and be available in appropriate languages.
- b. Measures taken when working with dead or diseased cervids should be the responsibility of National authorities. Measures that have been applied in North America during hunting should be re-evaluated when sufficient data are available.

6.2.3 Food safety (ToR 1.4)

- a. As only meat from healthy animals should be considered fit for human consumption, the risk linked to consumption of CWD-infected animals should be very low. This opinion includes meat from the 2016 hunting season and previous seasons.
- b. Human exposure through berries, other plant materials, or soil is considered to represent a negligible risk.

6.2.4 Surveillance in humans (ToR 1.5)

Prion diseases are under constant surveillance by health authorities. This system should be adequate for follow-up of any cases suspected of having exposure to cervids in Norway.

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Appendix I

Table 5. Prion diseases in various species.

Species	Name of disease	Disease mode	Comments
Human	Sporadic Creutzfeldt-Jakob disease (sCJD)	Sporadic	Approximately 1 case/million population per year. Similar appearance globally.
	Genetic Creutzfeldt-Jakob disease (gCJD)	Familial	Extremely rare
	Iatrogenic Creutzfeldt-Jakob disease (iCJD)	Medical or surgical treatment	Extremely rare
	Variant Creutzfeldt-Jakob disease (vCJD)	Infection, probably oral route	230 cases recorded globally. In the United Kingdom 178. Linked to intake of BSE contaminated food-stuffs. At least three cases related to blood transfusion.
	Gerstmann-Sträussler-Scheinker syndrome (GSS)	Familial	Extremely rare
	Fatal Familial Insomnia (FFI)	Familial	Extremely rare
	Kuru	Foodborne, cannibalism	Eradicated
	Variable Protease Sensitive Prionopathy (VPSPr)	Sporadic	Extremely rare

Species	Name of disease	Disease mode	Comments
Bovines	Classical bovine spongiform encephalopathy (BSE), seen in farmed cattle and captive bovines in zoos.	Foodborne	Major epidemic with more than 200 000 cases, mainly in Great Britain and Western Europe, a few cases in the USA, Canada and Japan
	Atypical BSE H-form, farmed cattle	Probably sporadic	Very rare, one case diagnosed in Norway 2015.
	Atypical BSE L-Form, farmed cattle	Probably sporadic	Very rare
Ovine and caprine	Classical scrapie, sheep and goat	Infectious, probably oral transmission	Wildly distributed, strong genetic modulation of occurrence.
	Atypical/Nor98 Scrapie, sheep and goat	Probably sporadic	Seen globally, also Australia and New Zealand
Mustelids	Transmissible Mink Encefalopathy (TME), mink	Probably foodborne	Probably derived from ruminant
Felids	Feline spongiform encephalopathy, Domestic cat and captive felids like tiger, cheetah, lion, puma	Foodborne	BSE contaminated foodstuffs

Species	Name of disease	Disease mode	Comments
Cervids	Chronic Wasting Disease (CWD), observed in white-tailed deer, mule deer, elk and moose. Diagnosed for the first time in wild reindeer in Norway March 2016.	Infectious	Until recently observed only in North America. Observed in South Korea after import from North America. Diagnosed for the first time in Europe in a wild reindeer in Norway March 2016.

Appendix II

Research

The Norwegian School of Veterinary Science (NVH), now faculty at the Norwegian University of Life Sciences, and the Norwegian Veterinary Institute (NVI) have for more than 20 years co-operated in various areas of prion research, particularly related to sheep scrapie. In Norway, prion research has largely taken place in the veterinary sciences and public health sector, with the two above-mentioned institutions as the main actors. This research has an international character with partners in many countries, particularly in Europe. The following paragraphs will briefly review some topics Norwegian prion researchers have investigated.

Isolation facilities allowing inoculation experiments with large animals as well as with laboratory rodents have been established and actively utilized in two localities, Sandnes and Oslo. Prion research at NVH has covered a broad range of scrapie related topics: diagnosis (clinics), genetics, pathology and pathogenesis, but also basic investigations into the physiological roles of PrP. At NVI, as a Governmental reference laboratory, prion research has focused on epidemiological investigations (Hopp et al., 2006), diagnostics (Benestad et al., 2008; Benestad et al., 2003), infectivity studies (Andreoletti et al., 2002; Lacroux et al., 2008), characterisation of prion strains (Arsac et al., 2007; Le Dur et al., 2005) and investigations of species barrier by bioassay in transgenic mice (Cassard et al., 2014).

Sheep scrapie was diagnosed in two rams imported from England in 1958. These were kept in an isolation facility and scrapie did not reappear in Norway until 1981, when the first outbreak in indigenous sheep was detected. A series of single flock outbreaks occurred during the 80s and early 90s, until eight flocks were diagnosed with scrapie in 1995 and 31 flocks in 1996. This increase coincided with the massive awareness related to BSE and the appearance of vCJD, and led to substantial increase in funding of scrapie research in Norway.

A diseased sheep with clinical signs unusual for classical scrapie was detected in 1998. This animal carried a PrP genotype that was rarely seen in classical scrapie and, interestingly, flocks-mates aged 5-6 years with PrP genotypes considered highly susceptible were observed (Tranulis et al., 1999). Based on differences in clinical symptoms, distribution of prion protein in the brain, molecular profile on Western Blot and genotype a new type of scrapie was described named Nor98 scrapie, later atypical scrapie (Benestad et al., 2003).

In parallel, a series of experiments involving oral inoculation of sheep were initiated, characterizing the uptake and subsequent amplification of prions in the gut-associated lymphoid tissues (Heggebo et al., 2003a; Heggebo et al., 2002; Heggebo et al., 2000; Heggebo et al., 2003b). These studies led to the development of an *in vivo* method for analysis of PrP^{Sc} presence in gut lymphoid tissue using rectal biopsies (Espenes et al., 2006b)

and gene expression analysis of laser-dissected tissue from the gut wall (Austbo et al., 2007; Skretting et al., 2004), as well as prion uptake studies (Jeffrey et al., 2006; Piercey Akesson et al., 2012; Press et al., 2004; Sorby et al., 2009).

The first cloning and characterization of the prion-like protein Doppel (Dpl) was also reported from Norwegian scrapie researchers (Comincini et al., 2001; Espenes et al., 2006a; Tranulis et al., 2001), as well as ultrastructural alterations in scrapie (Ersdal et al., 2009; Ersdal et al., 2003a) and investigations in flock outbreaks (Ersdal et al., 2003b). In collaboration with partners at Roslin University, Edinburgh, further studies of genetic predictions related to susceptibility towards prion disease, including that of carnivores have been performed (Campbell et al., 2013; Stewart et al., 2012).

Studies addressing the physiological roles and cell biological properties of PrPC have also been actively pursued (Lund et al., 2009; Lund et al., 2007; Tveit et al., 2009; Tveit et al., 2005). This has recently been developed further by use of a unique line of Norwegian Dairy Goats that due to a nonsense mutation lack expression of PrP (Benestad et al., 2012; Reiten et al., 2015).

Conclusion: Norway has for more than 20 years been actively engaged in prion research, particularly within the veterinary sciences. This contributes experience and adequate competence to support Governmental bodies in handling CWD and other prion diseases occurring in Norway.