

THESIS

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Chronic Wasting Disease Outbreak in Scandinavia

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List of abbreviations

BSE: Bovine spongiform encephalopathy

CJD: Creutzfeldt-Jakob disease

CNS: Central nervous system

CWD: Chronic wasting disease

DNA: Deoxyribonucleic acid

EFSA: European Food Safety Authority

ELISA: Enzyme-linked immunosorbent assay

FFI: Fatal Familial Insomnia

FSA: Food safety authority

GALT: Gut-associated lymphoid tissue

GSS: Gerstmann-Sträussler-Scheinker disease

IHC: Immunohistochemistry

kDa: kilodalton

LRS: Lymphoreticular

NINA: Norwegian institute for nature research

NVI: Norwegian veterinary institute

OIE: World organisation for animal health

PCR: Polymerase chain reaction

PK: Proteinase K

PRNP: Prion protein gene

PrP: Prion protein

PrP^c: Cellular prion protein

PrP^{Sc}: Scrapie prion protein

PSU: Primary sampling units

RAMALT: Rectoanal mucosa-associated lymphoid tissues

RNA: Ribonucleic acid

RT-QuIC: Real-time quaking-induced conversion

TSE: Transmissible spongiform encephalopathy

vCJD: variant Creutzfeldt-Jakob disease

VKM: The Norwegian scientific committee for Food and Environment

WB: Western blot

1. Introduction

Chronic wasting disease (CWD) and scrapie are so-called prion diseases, or transmissible spongiform encephalopathies (TSEs) affecting cervids and small ruminants respectively. These are diseases where the infective agent are misfolded prion proteins which accumulate in the brain, giving signs consistent with neuronal damage, as ataxia, emaciation, behavioural changes etc. Both CWD and scrapie are considered the most contagious of the prion diseases, giving major outbreaks in herds where the diseases are present, challenging farmers and the national authorities both concerning economics and animal welfare.

CWD was for the first time detected in Europe, when a wild reindeer in Norway succumbed to the disease in 2016. The disease has been familiar in the US since the 1960s and has been spreading over the Northern American continent during the last decades. South-Korea has also had confirmed cases of CWD in deer, but these was quickly connected with import of Canadian elk. Since the discovery of a cervid TSE on European soil, surveillance and control programs have become an important factor in countries with cervid populations.

Norway decided to cull the entire reindeer herd in the area where the first diseased reindeer was found and has used major resources on the surveillance and testing of other cervids in the country. A sanitation and quarantine plan are made for the area, in addition to a reintroduction strategy for when it is considered safe to establish a new wild reindeer population in the area. A new, novel type of CWD was also discovered in Eurasian moose and in red deer in Norway the same year, as new knowledge of the disease made the people more vigilant and sent self-dead or euthanized diseased cervid animals for testing for CWD to the Norwegian Veterinary Institute (NVI). This discovery did again put the authorities handling the Norwegian wild-animal populations on high alert, as the diseased moose was discovered in an area far from where the reindeer herds were situated. The neighbouring countries Sweden and Finland have also increased testing on their cervid population and has indeed the last couple years confirmed the atypical type in their moose-population. Research projects and testing are still ongoing, and new cases of the atypical type are sporadically detected.

The NVI launched several research projects in cooperation with national and international science communities, and several of these were thoroughly researched before the choice of

the topic for this thesis. A comprehensive literature review was initiated to answer the following questions:

- I. Are there any similarities between CWD and other prion diseases, here with scrapie as examples?
- II. Are outbreaks of prion diseases handled differently by the authorities when they are affecting wild vs. domestic ruminants, here with CWD in reindeer and scrapie in sheep as examples.
- III. How does the common folks respond to the authorities' decisions and measures taken when handling CWD in reindeer and scrapie in sheep?

The basis on the choice of the topic for this thesis was the announcement of the discovery of CWD in Norwegian wild reindeer and later, the novel type in moose. The choice to compare with scrapie was because these prion diseases are relative similar and there have now been cases of both in Norway. It is interesting to compare how the government handle prion diseases differently in wild and domestic ruminants, and how the people answer to the measures decided. Answers on these questions could be helpful to better understand the epizootiology of the disease or make it easier to decide which response and what control measures to take in such outbreaks.

2. Materials and methods

The information used in this thesis are accessed through published materials online. A review was conducted that mainly used the search engines “Google Scholar” (scholar.google.com) and “Pubmed” (ncbi.nlm.nih.gov/pubmed), where various combinations of the key words “chronic wasting disease”; “Prion diseases”; “Norway”; “Scrapie”; “Sheep”; “Reindeer”; “Cervids” etc. were used, in addition to more specific search combinations when a narrower topic were researched, e.g. “Nor98 sheep Benestad”, when more information about the atypical scrapie was researched, and as Sylvie Benestad was the scientist known to publish the first article about this disease. To select the most relevant articles, primarily the titles and the published year was the most important criterion, further the abstracts on these selected articles were screened. For the most, articles published in English or Norwegian in the period from 2010 till 2019 were used, even though specific older articles and reports were necessary to use, to get a sufficient overview of the topics discussed. Approximately 10-15 articles obtained from the primary search were selected for further review on the topics relevant to the thesis. Cited articles used in these initially selected articles were later used to further investigate specific topics mentioned in the review articles.

In addition, several reports, plans and articles published by the Norwegian Veterinary Institute (vetinst.no) and the Norwegian Food Safety Authorities (mattilsynet.no) were used on the topics related to the prion-disease outbreaks in Norway. The reports published after the discovery of CWD in Norway - “CWD in Norway” (Tranulis et al., 2016) published by the VKM and “Surveillance of CWD in Norway in 2016 and 2017” (Rolandsen et al., 2018) and “The surveillance programme for CWD in free ranging and captive cervids in Norway 2018” (Våge et al., 2019) written and published by the NVI were mostly used regarding the sections about CWD in Norway. The webpage managed by the NVI on CWD statistics (vetinst.no/skrantesykestatistikk) were visited several times to follow the development on the disease in real-time.

The annual control and surveillance reports on scrapie in Norway published by the NVI during the years 2000-2018 were used for chapter 3.6, in addition to an article published in the Norwegian Journal of Veterinary medicine in 2000 titled “[Scrapie in sheep in Norway, history and epidemiology](#)” (transl. from Norwegian) which thoroughly summarised the classical scrapie outbreaks in Norway, measures laid and research done.

For the measures laid down after both disease outbreaks, regulations published on the Norwegian FSA were used, accessible from https://www.mattilsynet.no/dyr_og_dyrehold/dyrehelse/dyresykdommer/skrapesjuka/ and https://www.mattilsynet.no/dyr_og_dyrehold/dyrehelse/dyresykdommer/skrantesjuka_cwd/

In all, approximately 90 articles, reports, webpages and books have been reviewed more or less during the work of this thesis. Not all have been cited in the final version but used in the initial phase to get an overview of the topics.

3. Results

3.1. Prions and prion diseases

The Nobel Prize winner and neurologist Stanley B. Prusiner defined prions as “*Proteinaceous infectious particles that lacks nucleic acid*”. A non-infectious, normal cellular prion protein is denoted as PrP^C, while the infectious, misfolded isoform of the prion is called PrP^{Sc} (PrP^{Sc}), no matter what species or origin of the disease.

Prions are proteins; hence they consist of polypeptide chains, and a structural transition of these, and major changes in the PrP's physicochemical properties lead to the conversion from normal cellular prion protein to the infective scrapie PrP. The refolding of the protein's structure is the reason they are folded differently, which give them different conformations. PrP^C is rich in α -helixes (approx. 40%) and has a small amount of β -sheet, PrP^{Sc} consist of 30% α -helixes and 45% β -sheet (Prusiner et al., 1998).

The multiplication of disease-associated prions (PrP^{Sc}) is initiated by the recruitment of normal cellular prion proteins (PrP^C) and stimulating its structural transition to the disease-causing isoform, accompanied by major changes in the physicochemical properties of the PrP. This change is demonstrated by the scrapie Prion protein's resistance to proteases and its inability to be solved in nondenaturing detergents (Prusiner, 2001; Prusiner et al., 1998).

Prion diseases are a family of rare and fatal progressive neurodegenerative diseases, characterised by incubation periods up to several months or years, spongiform changes seen as neuronal loss, and no inflammatory response, commonly seen in more frequent viral and bacterial diseases, are visible in blood or cerebrospinal fluid (U.S. Department of Health & Human Services, 2019a). The species naturally susceptible to this kind of disease are usually a narrow spectrum of mammals, namely humans and ruminants. Captive carnivores have also been diagnosed with prion diseases, these animals contract the disease orally, through feedstuffs contaminated with prions. Prion diseases can also be experimentally transmissible, Transmissible Spongiform Encephalopathy is therefore another used collective term for the diseases (Tranulis et al., 2016).

The most known prion diseases are chronic wasting disease (CWD) in cervids, scrapie in sheep and goat, and bovine spongiform encephalopathy (BSE) in cattle. Creutzfeldt-Jakob disease (CJD) and the variant form (vCJD), Gerstmann-Sträussler-Scheinker (GSS) disease, Fatal Familial Insomnia (FFI) and Kuru are prion diseases occurring in humans.

Prion diseases are caused by a toxic gain of function in a normal host cell protein, they induce the atypical folding of specific normal cellular proteins. The manifestation of prion diseases is a result from the accumulation of PrP^{Sc}, the failure to clear this infectious protein from the brain, where they are richest in number, is the direct cause of the neuronal damage (Collins et al., 2004; U.S. Department of Health & Human Services, 2019a).

The clinical manifestations of transmissible spongiform encephalopathies may differ tremendously and are non-specific. In humans it most common manifests as dementia and with deviant behaviour, but ataxia, insomnia, paraesthesia and paraplegia are also common clinical signs. In animals, the signs manifest as a result of the pathological changes in the nervous system, causing neurological disturbances as ataxia, pruritus and scratching, loss of body condition, hyperexcitability etc...

The areas of the CNS most heavily affected are the medulla oblongata and diencephalon. Pathological findings of the CNS vary from no atrophy to widespread atrophy, from marginal to widespread neuronal loss, from sparse to extensive vacuolation or spongiform changes, from mild to severe reactive astrocytic gliosis, and from the total absence of PrP amyloid plaques, to a great quantity of plaques. None of these findings, only the presence of PrP amyloid plaques is a pathognomonic feature for a prion disease (Prusiner, 2001)

Prion diseases can be horizontally transmitted between susceptible individuals or transmitted via food, or iatrogenic route. CWD and scrapie disease are prion diseases considered highly infectious and display considerable in vivo dissemination, resulting in facile transmission between susceptible animals. BSE and CJD on the other hand, have limited to no direct transmission from one individual to another (Gough and Maddison, 2010).

Of the acquired TSEs, the oral route is the most common way of infection. After *per os* intake, the infective prion accumulates and amplifies in the gut-associated lymphoid tissues (GALT), the prion then spreads to other lymphoreticular (LRS) tissues, and to the enteric nervous system, from where it is finally spread and infect the central nervous system. Within the CNS the prions replicate and spread centrifugally via the peripheral nervous system to other sites for secondary prion replication (Gough and Maddison, 2010; Press et al., 2004).

As prion diseases can be transmitted under experimental conditions, there is a theory that this type of diseases might be present in a wider variety of animals than currently recognized, and the species that do not develop signs can be silent carriers. For example, the BSE agent is

known to be able to infect several species, including cats, goats and humans via the food chain. The intake of BSE-infected meat is now known to be the cause of variant Creutzfeldt-Jakob disease (vCJD) in humans, although there are millions of people that have consumed BSE contaminated meat, there are few known cases of the vCJD. This indicate that there is a very low transmission rate for BSE to humans, this poor transmission of diseases between species is a phenomenon referred to as the species barrier (Gough and Maddison, 2010; Tranulis et al., 2016).

The disease can be manifested as infectious, genetic or sporadic disorders. Human prion diseases for example, are not considered contagious under normal conditions. They are most commonly sporadic, caused by a spontaneous misfolding of the PrP^C into its disease-causing conformer PrP^{Sc} (Prusiner, 2001; Tranulis et al., 2016).

3.2. Chronic Wasting Disease

Chronic wasting disease (CWD) is a fatal, neurodegenerative prion disease diagnosed in the family of Cervidae, including captive and free-ranging deer, elk, moose and reindeer in North America, South Korea, and recently in Scandinavia.

When a substantial quantity of captive mule deer (*Odocoileus hemionus*) in Colorado, US during the late 60s, developed neurological signs, had behavioural changes and a significant loss of weight, their keepers started to recognise the signs and an investigation to determine the cause was started. Initially, it was thought to be caused by the stress of captivity, nutritional deficiencies or an intoxication, but as further examinations were done, it became recognised as a new TSE (Tranulis et al., 2016). During the first couple decades after it first was diagnosed, it was thought that the prion disease only affected mule- and black-tailed deer held in captivity in the western states of Colorado and Wyoming (Williams and Young, 1980). In the early 1980s it was discovered in wild deer and elk (*Cervus canadensis*), and since 2000, the United States areas which has confirmed cases of CWD in free-ranging cervids has expanded to at least 24 states, including states in the Mid- and Southwest, and some areas on the East Coast (U.S. Department of Health & Human Services, 2019b). In addition, CWD is also confirmed in deer and elk in the provinces of Alberta, Saskatchewan and Quebec in Canada (Government of Canada, 2014) and it has been observed in red deer (*Cervus elaphus*) and sika deer (*Cervus nippon*) in South Korea, after import of elk from Canada (Våge et al., 2019).

This widespread occurrence of CWD led to a surge in CWD research, which focused on understanding species susceptibility, transmission and pathogenesis, spatial epidemiology, diagnostic tools, strains, and cervid PrP structure. Still, there are several aspects about the diseases not fully understood (Sigurdson, 2008).

Chronic wasting disease is one of the few TSEs considered highly infectious and are believed to be horizontally transmitted. Pathogenesis studies of the disease have revealed that there is a massive deposition of PrP^{Sc} in the CNS and extraneural tissues, like lymphoid tissues, endocrine glands (i.e. pancreas, adrenal gland) and skeletal muscle (Sigurdson, 2008). Studies of experimental models and natural infections have shown that scrapie and CWD agents are both excreted and secreted in biological materials associated with parturition, specifically with milk and placenta. Urine, faeces, saliva and even skin-samples have been revealed to contain excreted of CWD prions. The placenta contains the highest levels of prion infectivity and seems to be an apparent source of post-partum transmission to the rest of its flock. It has also been proven that milk contain biologically relevant amounts of the disease agent, while levels of prion within excreta such as urine, faeces and saliva are very low, but are produced throughout long periods of preclinical disease as well as during clinical disease (Gough and Maddison, 2010).

Experiments have revealed that CWD is transmissible to a wide variety of cervids and a range of non-cervid species are susceptible to the prion disease by intracerebral inoculation, e.g. sheep, goat, cattle, cats and some rodent species. The experimental transmission of CWD to other mammals *per os* is far less efficient, demonstrating that the species barrier under natural conditions is rather high. It has been possible to experimentally infect reindeer by oral challenge, while the North American subspecies, known as caribou (*Rangifer tarandus*), have not been diagnosed with CWD (Benestad et al., 2016).

As other prion diseases, the incubation period for CWD is long – on average it varies from two to four years (minimum 16 months), therefore the disease is exclusively found in older animals. The duration of the disease, however, is rather short. In most cases, the diseased animal dies within four months after the onset of clinical signs, but can vary from a few days and up to a year (Tranulis et al., 2016). Environmental stress, for instance periods of extreme cold, difficulties to find sufficient forage and water, predators, etc. might shorten the length of clinical disease. Because of the increased stress in the wild population compared with cervids

held in captivity, the clinical duration is likely to be shorter in free-ranging cervids (Williams, 2005).

The clinical signs of CWD are non-specific and might not always be apparent. It is usually manifested as emaciation and altered behaviour, such as listlessness, isolation from the herd, lowered head and ears, repetitive walking and hyperexcitability. Polyuria, polydipsia, grinding of the teeth, frequent regurgitation and sialorrhoea are common signs during the terminal stages of the disease. Secondary aspiration pneumonia is quite common, possibly due to decreased swallowing function. Stress may trigger the appearance of clinical signs and can lead to unexpected mortality. CWD do, without exception, end with death (Benestad et al., 2016; Vikøren et al., 2016).

The post-mortem lesions in CWD, as in the other animal TSEs, are non-specific and those present reflect the clinical signs: rough and dry hair coat not appropriately shed, megaesophagus and may or may not be of poor body condition, depending on stage of clinical disease. The animals are usually emaciated, often have watery and frothy rumen contents and, if the water supply have been sufficient, they have diluted urine in the terminal stages of the disease. If water supply has been sparse, as they usually are in wild populations in wintertime, the animals are usually dehydrated (Williams, 2005).

During histopathological examinations of animals suspected diseased of CWD, only the Grey matter of the CNS can show lesions. The anatomic distribution of lesions in the CNS is fairly constant, they are bilaterally symmetrical and the alterations in the spongiform tissues are discernible. Vacuolization occurs in neuronal perikarya and neuronal processes, less conspicuous features are neuronal degeneration and astrocytic hyperplasia and hypertrophy. The most outstanding lesions are found in the diencephalon, olfactory cortex, and nuclei of the medulla oblongata and as in all other prion diseases, there are no inflammatory cell response existing, except if secondary infections are present. Amyloid plaques are common and emerge as slightly pale, fibrillar eosinophilic areas of neuropil in haematoxylin and eosin stained preparations, surrounded by vacuoles (“florid plaques”). In animals visible affected of CWD, a well-fixed and prepared section of the medulla oblongata at the level of the obex is satisfactory for the final diagnosis, and was used for the surveillance of CWD before testing with immunohistochemistry (IHC) became available (Williams, 2005).

As the clinical signs of CWD are non-specific and often hard to determine, diagnosis based on these are difficult and not accepted by the OIE. Today, CWD testing are done post-mortem on

tissue from the brain stem. A few ELISA-based rapid tests are validated for the detection of CWD prions, but in cases where the results obtained from these rapid tests are inconclusive or positive, confirmatory examinations by either immunohistochemistry or western blot methods and protocols as laid down in the latest edition of the Manual for diagnostic tests and vaccines for Terrestrial Animals of the OIE are mandatory. If none of these methods are able to confirm the positive result obtained in a rapid test, an adequate quantity of the tissues used must be sent to the EU Reference laboratory for confirmatory testing to be undertaken (Cassar, 2017).

In the US, IHC methods on biopsies of rectoanal mucosa-associated lymphoid tissues (RAMALT) are becoming more common to use in the surveillance of CWD in the cervid population, as these are tissues that can be collected perimortem (Benestad and Telling, 2018; Keane et al., 2009)

Recent studies performed in American elk revealed that ante-mortem samples of RAMALT biopsy specimens and, to a lesser extent, nasal brushings of the olfactory mucosa, analysed with so-called RT-QuIC (real-time quaking-induced conversion) method have shown to be a very rapid and sensitive screening method for the detection of CWD in cervids, although further validation is needed before the method is accepted for confirmation. RT-QuIC amplifies misfolded proteins, similar to how PCR methods amplifies DNA and RNA (Haley et al., 2016; Manne et al., 2017).

3.3. CWD outbreak in Norway

3.3.1. First confirmed CWD cases

In March 2016, Chronic Wasting Disease was for the first time diagnosed in Europe. A female reindeer from the wild reindeer area called “Nordfjella region zone 1” died during an attempt to chemically immobilise wild reindeer in Norway for the purpose to place a radio-collar on them, as a part of another study (Tranulis et al., 2016). Nordfjella is an approximately 3000 km² mountain area in the southern part of Norway. The wild reindeer herds residing in this area are divided into two subpopulations, due to the influence by hydroelectricity developments, roads and recreational cabins in the area. The Nordfjella populations are divided into zone 1 and a zone 2, situated respectively north and south of the highway going through the area (Hansen et al., 2017). The infected reindeer were found recumbent after the research crew searched for a missing dart in the area not far from the herd. She still had eye reflexes and was able to move her limbs but was exhausted with froth

coming from her mouth and an elevated body temperature (41,9°C). She died not long after, and since she had died of natural causes, the scientists could without further notice take her down for a post-mortem investigation, as no ethics approval was required. It was estimated that the reindeer was an adult between 3-4 years old from looking at eruption and wear of the teeth (Benestad et al., 2016).

The necropsy revealed that the reindeer's body condition was poorer than it normally would have in a non-pregnant female reindeer during that time of the year, and there were minor areas of hair-loss on the chest, elbows and thighs. The major pathological findings, however, were several haemorrhagic and ruptured skeletal muscles, with the hind limbs worst affected, general congestion and lung oedema. Tissue samples from the brain, heart, lungs, liver, kidneys, skeletal musculature and tracheobronchial lymph nodes were taken out, fixed and prepared for further analysis. Histological investigations revealed acute degeneration of skeletal musculature and in the brain, examination of the obex showed vacuolation, especially in the dorsal motor nucleus of the vagus nerve, in the neuropil and in neurons. For the detection of abnormal prion proteins, the medulla oblongata was analysed using a commercially available ELISA test. As this test came out positive, control investigations were done using a commercially available Western blot test, its resistance against proteinase K and immunohistochemical labelling. The presence of PrP^{Sc} were detected both in the brain tissues and in the tracheobronchial lymph nodes, and confirmed by the OIE reference laboratory for CWD in Canada (Benestad et al., 2016).

In May and June the same year, Chronic wasting disease was diagnosed in two Eurasian moose (*Alces alces*) approximately 300 km north of the first confirmed case, in Trøndelag county in central Norway (Tranulis et al., 2016). In the first case, the moose was emaciated and showed no fear for humans, in the second case in 2016, the moose was found dead in a river. Post-mortem investigations revealed that she was pregnant, with normal body condition and cause of death was trauma. In 2017, a third case of CWD in moose in the same area was discovered. This animal demonstrated abnormal behaviour, showing reduced fear of humans. Necropsy of the moose revealed poor body condition and a severe dislocation of the left hip joint. The 3 moose were females, and based on dental analysis their ages were estimated approximately 13-14 years (Pirisinu et al., 2018).

CWD was for the first time discovered in red deer (*Cervus elaphus*) in Norway, October 2017 in the municipality of Gjemnes in mid-western Norway (Norwegian Veterinary Institute, 2019; Rolandsen et al., 2018).

3.3.2. Atypical CWD detected in Eurasian moose and red deer.

During the extensive investigations performed in Norway in 2016 and 2017, CWD was diagnosed in free-ranging reindeer, moose and deer. Characteristics with these findings made the investigating authorities suspecting there were at least two different types of the prion disease; classical and atypical (Novel) CWD. It has not been possible to distinguish the infective type found during the last decades in USA and Canada from the CWD-prions found in the reindeer in Nordfjella using the traditional diagnostic methods. Further studies however, using bioassays in mice, have revealed that there are some fine distinctions separating the Norwegian disease from the one found in North America, similar to differences found in other animal and human TSEs.

Analysis made in the 3 moose and the one deer tested positive for CWD further distinguish the CWD types found, as these animals showed a type different from both the North American type and the type found in reindeer in Nordfjella. The main differences between the typical and atypical CWDs are, as prions in the typical type can be detected in both nervous- and lymphoid tissues, they can only be found in brain sections in the atypical type (Rolandsen et al., 2018).

Immunohistochemical analysis of brain tissues from the moose established that they shared the same neuropathologic phenotype, mainly characterised by intraneuronal deposition of PrP^{Sc}. This was a pattern very different from the reindeer prions and has never been reported in CWD-infected cervids before. Additionally, further analysis using Western blot revealed a PrP^{Sc} type distinguishable from previous CWD cases and from other identified ruminant prion diseases in Europe (Pirisinu et al., 2018).

The moose and deer in the confirmed cases were old (13-16 years) and prions were only detected in the brain, while in contrast, in the reindeer in Nordfjella and in most North American deer-species with CWD, prions were also found in lymph nodes. For diagnosis of the atypical cases, analysis of brain tissues is necessary, but in the surveillance programmes in North America, several states only examine the lymph nodes. This is sufficient for the classical type of CWD, but cases of the atypical CWD can remain undetected (Rolandsen et al., 2018).

3.3.3. Reindeer in Norway

There are four cervid species common in free-ranging populations in Norway: Eurasian moose (*Alces alces*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and reindeer (*Rangifer tarandus tarandus*).



Figure 1: Norwegian Semi-domesticated reindeer during winter in the south-eastern part of Trøndelag county.

Foto: Sidsel T. Bakås, 2019.

The Norwegian free-ranging reindeer represent the last remnants of the wild tundra reindeer in Europe. It has existed in these areas since the end of the last ice age approximately 10 000 years ago. For thousands of years they lived in most alpine regions all over the country, but after the introduction of weapons used for hunting, they were almost extinct. Today, the surviving free-ranging reindeer live in the mountain areas in southern Norway (Handeland, 2014). There is approximately 25 000 animals in the winter population found in fragmented sub-populations in the remote alpine regions from the middle of the country (Trøndelag) to the south of Norway (Aust-Agder) (Benestad et al., 2016).

In addition to the free-ranging reindeer herds in the southern part of Norway, there are also a tradition for reindeer husbandry in the Sámi culture. Almost 40% of Norway's landmasses with the majority in Finnmark county, are used as pasture for reindeer in approximately 80 herding districts, stretching from Hedmark county in south to Finnmark in north. Some of the districts are sharing grazing areas with Swedish and Finnish reindeer herders across the borders. The herding usually has a semi-nomadic structure, in which the reindeer are herded/transported between winter pastures and the calving ground/summer pastures. The

animals in most districts are considered free-ranging and can go several days without inspection. In some periods, such as during the calving period, the animals can be left alone for longer periods to avoid disturbances. This is why, although the reindeer has owners, they are not considered tame animals, they are termed semi-domesticated reindeer (Tranulis et al., 2016).

Reindeer belongs to the order Artiodactyla and are in the family of Cervidae (deer-animals) and are widespread in the arctic and subarctic regions all over the world. There are seven remaining subspecies of reindeer, in mainland Norway however, all animals belong to the subspecies *Rangifer tarandus tarandus*, although there is another subspecies which are managed by the Norwegian government on the island of Svalbard. The Svalbard reindeer (*Rangifer t. plathyrhynchus*) only exist on this small island and consist of approximately 10-12 000 animals (Tryland and Thoresen, 2014).

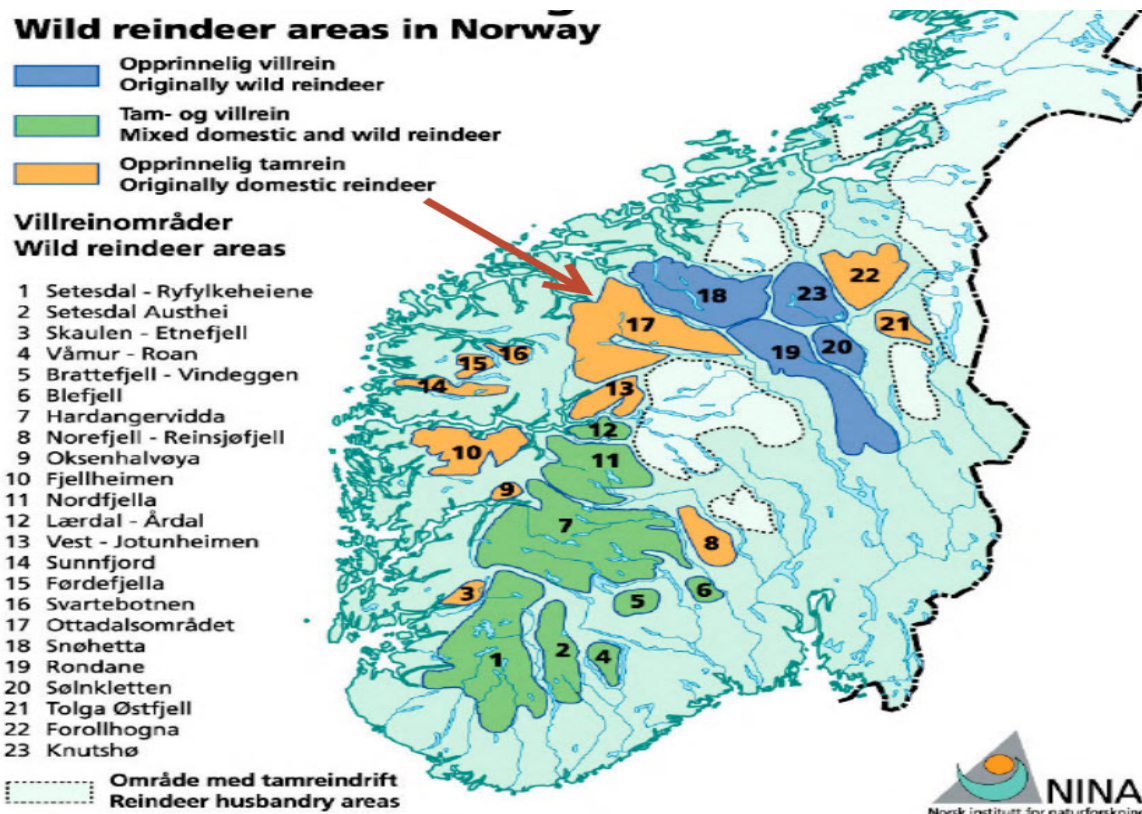


Figure 2: Distribution of the 23 different populations of wild reindeer (*R. t. tarandus*) in southern Norway. Nordfjella, marked as number 11 is the only area where the classical type of CWD is detected (Norsk villreinsenter, 2019).

3.3.4. Response from the authorities

VKM (The Norwegian Scientific Committee for Food and Environment) presented a report in March 2017 where they recommended to cull the entire reindeer herd in Nordfjella zone 1, follow through a fallowing of the area and eventually restocking from a healthy population. The report also recommended other measures, like remove all licking stones for wild deer animals and domestic ruminants grazing in the area, redevelop the ground around existing licking stones and reduce the cervid population where CWD were confirmed and in the neighbouring areas.

The Norwegian Food Safety Authority (FSA, Mattilsynet), together with the National Veterinary Institute, the Norwegian Environment Agency and the Norwegian Institute for Nature Research (NINA) concluded in a letter to the Ministry of Agriculture and Food in April 2017 that these recommendations to prevent the spread of CWD should be followed, and within reasonable time, the entire reindeer herd in Nordfjella zone 1 should be culled. The Ministry of Agriculture and Food agreed with these recommendations and delegated to the Food Safety Authority and the Environment Agency to work out a concrete plan for the reindeer cull. This plan should include measures so that there was no reindeer left in Nordfjella zone 1 by 1st of May 2018, organising the euthanasia and hunting as effective and gently as possible, having a quarantine period as short as possible, with an emphasis on the animal welfare. Further progress, sampling, meat and waste handling and infectious preventive measures should be described. A plan on how the wild reindeer population should be reintroduced after the planned fallow period should also be described, including the involved ground keepers, farmers and affected municipalities (Mattilsynet and Miljødirektoratet, 2017).

As reindeer are considered the most gregarious of the cervid population, and CWD is highly contagious, their wandering could easily spread prions to other herds and deer animals. Therefore, the drastic decision to cull the entire herd was decided on the basis of only 3 positive samples. The slaughter started already in August 2017 by amateur hunters hunting by ordinary quota, which sent brain and lymph nodes to the Norwegian Veterinary Institute for testing and was welcome to use the meat if the samples came back negative. The aim was to harvest 800 out of the 2000 reindeer during this period, but for various reasons, among others due to a resistance against the reindeer cull among the locals in the area, only 582 reindeer were killed. After the ordinary hunting period, professional marksmen from the Norwegian Environment agency were used to cull the rest of the herd residing in the northern part of Nordfjella wild reindeer area. The last known herd was removed on February 25 and after this

point, only a few solitary animals were left. In total, 2024 reindeer were removed and sampled in the period from August 2017 till May 2018. Out of these, 19 tested positive for the prion disease, indicating that the reindeer cull were initiated at an early epidemic stage (Mysterud and Rolandsen, 2018; Stokstad, 2017).

To decide the duration of the quarantine period, it is necessary to have an ongoing overview on the infective agents in Nordfjella zone 1. Based on today's knowledge, this period is set to last minimum 5 years from the last wild reindeer is assumed removed from the area.

New regulations were made considering licking stones (mineral stones) placed in the Nordfjella area for sheep grazing during the summer. The area surrounding these stones can be major sources for infective prions, as these licking stones are used by wild cervids and ruminants in the same area. As the prions are shed by saliva, faeces and urine and can persist in the environment for long times, these places were considered one of the most important risk factors for the spread of CWD. The sheep using the licking stones can potentially carry prions and indirectly transmit the disease to deer species in the same area. Based on this, several licking stones in the zone were removed, but most were enclosed with special barriers, only letting sheep through (Mattilsynet and Miljødirektoratet, 2017). A new regulation was implemented during the summer 2019 regarding the division of zones in the Nordfjella area. A total ban against licking stones in the Nordfjella area was implemented, closing off the enclosures built for the sheep and existing licking stones had to be removed. Only already existing sheep stocks was allowed to graze in the area, banning the introduction of new animals (Landbruks- og matdepartementet, 2019).

Following the discovery of CWD in Norway, The Norwegian Food Safety Authority initiated an improved and expanded surveillance of the disease. There was an existing passive surveillance programme on CWD in the country, where approximately 2200 cervids were tested in the period from 2004 till 2016. After CWD were confirmed, massive efforts were made to collect brain and lymph node samples from cervids, where especially hunters were involved and thought how to collect and handle these materials and send them for analysis at NVI. The wild reindeer areas around Nordfjella were especially targeted, and the hunting quotas were increased to reduce the density and get a sufficient number of samples to rule out the disease. Other cervids were also tested, and the surveillance programme were also implemented into the slaughterhouses receiving semidomesticated, farmed and wild cervids for processing. As the atypical type was discovered in the town of Selbu in Central-Norway, increased surveillance was implemented in the neighbouring areas. From 2016 and till

October 2019, 92724 samples have been analysed where there was positive results in 19 reindeer, 6 moose and 1 deer (Norwegian Veterinary Institute, 2019).

Other measures introduced was the ban against natural deer-urine lures, it was forbidden to transport live cervids across the national border, if semi-domesticated reindeer was to be moved across county borders, approval from the FSA was needed, and it was not allowed to move other deer animal species between counties. The obligatory notification to the authorities in case of observation of diseased cervids was also implemented (Mattilsynet, 2018a).

The Norwegian Veterinary Institute launched several research projects in the early phase as the prion disease were identified, focusing around four main problems with CWD: The difference between the “moose type” and the “reindeer type”, epidemiology, establish a CWD-test on live cervids and a mapping on the genetic sensitivity in Norwegian cervids (Veterinærinstituttet (Norwegian Veterinary Institute), 2016).

Most of these projects are still ongoing, but there have been published some results and theories. How CWD first came to Norway and was able to infect wild reindeer was one of the first questions the researchers had to assess. A detailed assessment on the probable route of introduction is still not clear, therefore only potential routes are discussed. The most likely route is that it somehow has been introduced from North America, where potential CWD infected lures or equipment have been brought to Norway. Among others, import of deer or mouse urine lures for hunting purposes and prion-contaminated equipment, clothing and footwear of hunters or other tourists were considered as potential routes. Also, exposure from other European countries, leading to the introduction to Norway have been a suggestion, particularly because Norway shares borders with Sweden, Finland and Russia. A theory that CWD may have existed undetected in the joint Fenno-Scandinavian cervid population for years and introduced to Nordfjella by migrating infective cervids from the neighbouring countries were proposed (Tranulis et al., 2016). The fact that Finland imported white tailed deer from North America in 1934 was also brought up, but as Finland had a major TSE testing program in the period between 2003 and 2015, especially targeting white tailed deer with no confirmed cases, this theory was considered unlikely (Benestad et al., 2016). Today, the most likely theory is the spontaneous occurrence of CWD, similar to sporadic CJD in humans, the atypical Nor98 scrapie type in small ruminants and the atypical BSE type affecting cattle, although further studies are needed (Tranulis et al., 2016).

3.4. CWD in the rest of Europe

In 2018 the first case of CWD was confirmed outside of Norway, in moose in Finland and a year later, in Northern Sweden, in March, May and September 2019 in three moose.

After the outbreak in Norway, other European countries intensified their surveillance programmes, there are surveillance ongoing in Estonia, where there in 2017 was 75 cervids tested, in Latvia where 100 primary sampling units (PSU) are defined, covering all the country, 1000 wild cervids are to be tested and 205 captive per year. Lithuania and Poland have also increased their surveillance on cervids, testing wild and farmed deer species nationwide.

Finland have defined 100 PSU and the surrounding areas where the positive moose case was discovered are subject to intensified surveillance, the same with its neighbouring country Sweden. They have defined each Sámi village and cervid farm as PSU, in addition to PSU covering the entire country for free-ranging cervids (European Food Safety Authority, 2018).

3.5. Scrapie disease

3.5.1. Classical scrapie

Classical scrapie is a fatal, neurodegenerative prion disease, a protein-misfolding disease affecting ovine and caprine species. The disease has existed for almost 300 years, as the first reported case was in Great Britain in 1732 in sheep, and further cases was described during the eighteenth and nineteenth century in the UK, Germany and other western European countries (Detwiler, 1992). For centuries there were different theories what caused the disease, and it was long discussed whether it was of genetic or infectious origin. The different propositions of what the TSE agent was ranged from a theory of sarcosporidia parasites in 1914, different virus types (filterable (1938), slow (1954)...), viroid and virion, to replicating polysaccharides (1966), proteins (1967) and spiroplasma species in 1979 (Schneider et al., 2008). In 1982, Dr. Prusiner published an article in the journal "Science", titled "*Novel Proteinaceous Infectious Particles Cause scrapie*". In this article Prusiner list a wide number of characteristics of the infectious agent causing scrapie distinguishing it from viruses, plasmids and viroids, concluding with it being an infectious protein, for which the 1997 Nobel prize winner (The Nobel Foundation, 1997) proposes the new term "prion". Special weight was laid on the agent's increased resistance to conventional protein denaturation treatments including ionizing radiation, UV light, heat and certain chemical substances (Prusiner, 1982).

Scrapie is a slowly progressing prion disease, usually affecting sheep between 2 and 5 years, or goats over 6 years old and the disease does affect both male and female animals equally (Mathiason, 2017). The incubation period can be up to several years, but there are a number of factors influencing the duration of this period (“Terrestrial Manual,” 2019). Incidence, susceptibility and incubation period of clinical disease in sheep following inoculation is controlled by host prion protein gene (PRNP) alleles. Particularly amino acid polymorphism at codons number 136, 154 and 171 are closely linked to relative susceptibility or resistance to scrapie in sheep breeds. The allelic variations commonly associated with susceptibility are results from amino-acid substitutions involving Alanine (A), Valine (V), Arginine (R), Histidine (H) and Glutamine (Q) (Greenlee et al., 2014). In cases where Valine (V) is present, instead of Alanine (A) on codon 136, the genotype becomes significantly more susceptible to scrapie, i.e. the VRQ/VRQ genotype commonly seen in Cheviot. In the ARR/ARR genotype, where Arginine (R) is present on codon 171 instead of Glutamine (Q), is highly connected with disease resistance. The survival and/or incubation time can be lengthened in cases where Histidine (H) is substituted for Arginine (R) at codon 154 (Houston et al., 2015). Based on this knowledge, efficient genetic breeding programmes have been introduced with a goal to eradicate and control scrapie. The goal is to eliminate and reduce the susceptible alleles (VRQ) and to promote the resistant allele (ARR) (Mathiason, 2017).

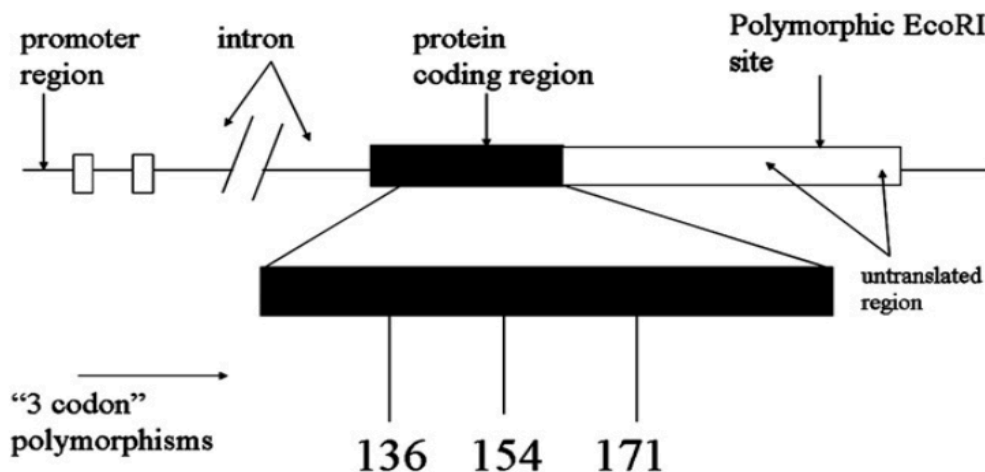


Figure 3: Diagram of sheep PrP gene structure showing the positions of polymorphisms discussed in the text, with the codons 136, 154 and 171, which are the major determinants of relative susceptibility or resistance (Hunter, 2007). E.g. if Alanine are present at codon 136, and Arginine are present on both codons 154 and 171, the resistant allele ARR is formed, however if Ala. is substituted with Valine at codon 136 and instead of Arg. at codon 171, Glutamine are present, the susceptible allele VRQ is formed.

As the disease affects the nervous system, the clinical signs presenting reflect the neuronal damage. In the early stages of the disease, the clinical signs are subtle and not always visible. They usually start with behavioural changes, presenting as confusion, the animal may become shy and more nervous, separate itself from the rest of the flock and sometimes it can become aggressive. As the disease progresses, the signs of a neurological illness become more prominent, and the diseased animal may show pruritus, ataxia, tremor, incoordination, head pressing and “star gazing”. The ataxia or the excessive scratching usually dominate, and the name “scrapie” is obtained from the scraping and rubbing against objects. This intense pruritus often results in major wool-loss, particularly over the chest, flanks, hind legs and on the base of the tail. In extreme cases, self-inflicted skin lesions may present in these areas (“Terrestrial Manual,” 2019; Detwiler, 1992). Eventually scrapie leads to recumbency and the disease is 100% lethal (Animalia Sauehelsenett, 2017).

Several studies have concluded that scrapie can be transmitted horizontally, and as especially high numbers of prions are excreted with foetal membranes during parturition, lambs are often infected at birth (Brotherston et al., 1968; Gough and Maddison, 2010; Hourrigan and Klingsporn, 1996; Vascellari et al., 2007). Studies have also revealed that sheep of susceptible genotypes can be infected vertically in utero with an ascending transmission from the placenta to the foetus (Spiropoulos et al., 2014), in addition to through milk and colostrum from the ewe to the lambs (Konold et al., 2013).

The scrapie prions have also been shown to be present in the salivary glands and on the tongue, hence it is believed to be shed in the saliva. The prions shed into the environment with the saliva will also contribute to horizontal transmitting the PrP^{Sc}, in addition, since the vast majority of the saliva produced by ruminants are swallowed, this can contribute to reinfection of the gastrointestinal tract tissues and further shedding with faecal materials (Tamgüney et al., 2012).

The prions can according to studies performed in the UK persist in the environment for a long time, studies have shown that scrapie agents can remain infectious for as long as 16 years (Georgsson et al., 2006), and has an extremely high resistance against disinfectants and detergents (Hawkins et al., 2015; Maddison et al., 2010). A study performed in 2015 uncovered that the scrapie prions can survive thorough decontamination regimes in a barn after an outbreak, as imported, scrapie negative but genetically susceptible sheep tested positive after residing in a pen where scrapie positive sheep had lived. The pens had been washed clean with pressure washer, decontaminated with high concentration sodium

hypochlorite solution, all moveable metalwork was either changed or re-galvanized and floors, walls and unmoveable steel were repainted with a hard-wearing floor paint, all according to recommended procedures. Personnel and equipment hygiene were strictly followed up and all the pens had separate entrances. Regardless of all these measures taken, 100% of the sheep tested positive for classical scrapie within 18 months after introduction (Hawkins et al., 2015).

Macroscopic post-mortem lesions are consistent with the clinical signs, and in sheep showing clinical disease wool less areas, often with skin lesions and general poor body condition can be consistent with scrapie. Mainly, pathological changes of scrapie in sheep and goats are limited to microscopic changes in the CNS. Histopathological lesions are predominantly found in the grey matter of the brain stem and include neuronal vacuolation and other forms of neuronal degeneration including cell loss, glial cell proliferation or hypertrophy in astrocytes (astrocytosis). The changes seen most is the cytoplasmic vacuolation of the neurons in the medulla, pons and mesencephalon, giving the typical spongiform appearance seen in prion diseases (TSEs). Amyloid plaques are also commonly seen in histological investigations of the CNS in scrapie diseased small ruminants (Detwiler, 1992; Dustan et al., 2008).

As scrapie is an OIE listed disease and considered a “List B”-disease in Norway, standardised diagnostic tests are available and described in the OIE Terrestrial Manual, together with recommendations concerning import and export from/to countries not considered free from scrapie, measures necessary in case of outbreaks and points needed to be fulfilled to be considered free from scrapie.

Scrapie is diagnosed on the basis of histopathological or IHC changes in the CNS and the detection and characterisation of resistant prion protein, using Western Immunoblot assays or Rapid tests based on WB or ELISA techniques.

With visualisation on a Western Immunoblot gel, classical scrapie is seen as immunolabelled bands corresponding to proteins with a range of molecular mass from 17 kDa to 27 kDa (“Terrestrial Manual,” 2019).

3.5.2. Atypical scrapie/Nor98

In 2003 a new, novel scrapie type was described for the first time after five cases of scrapie with unusual characteristics was diagnosed in Norway. The disease was detected in Norwegian sheep in 1998, hence its designated name “Nor98”. Nor98, or atypical scrapie,

was differentiated from classical scrapie on basis of its distribution only in the cerebellar and cerebral cortices, especially suspicious was the complete absence of lesions at the level of the obex. Prion proteins could not be detected in lymphoid tissues in these sheep, making it only detectable after a threshold level of PrP^{Sc} had accumulated in the brain. This atypical scrapie also seemed to infect genetic lines of sheep previously thought to be resistant against the classical small ruminant TSE and mostly older sheep were affected.

As only one animal in each of the five flocks euthanized and investigated on suspicion of scrapie tested positive, and none of these diseased animals previously had been in contact with herds with a history of scrapie, it was theorised that the Nor98-type occurred spontaneous, and was not as contagious as the classical type. Further investigations done supported this theory, because in addition to the geographical distribution, where Nor98-infected sheep occurred in areas with no previous history of scrapie, or no contact with other flocks with scrapie and the absence of major outbreaks in flocks where it was detected. The lack of PrP^{Sc} immunolabelling and histopathological changes in the obex may suggest that the route of entry for the scrapie agent, which in the classical type is via the dorsal motor neuron of the vagus nerve, might not apply in cases of the atypical Nor98-type (Benestad et al., 2003; Mathiason, 2017). Studies of the Nor98 agent's transmissible properties have revealed that experimental transmission to standard mice have not been successful, although the use of transgenic mice expressing ovine VRQ PrP have indeed been proven efficient, indicating that Nor98 is a truly infectious TSE agent (Benestad et al., 2008). A small study from 2007, where a cheviot sheep was inoculated intracerebrally with cerebellum from a case with confirmed Nor98. The sheep was euthanized 378 days after inoculation, presenting neurological signs, indicative of a diffuse brain disorder. WB analysis of the sheep's brain tissues revealed a pattern consistent with the Nor98-type, identical with the pattern the original donor-sheep showed. This was the first case where the Nor98-agent was successfully transmitted to another susceptible species (Simmons et al., 2007).

After the Nor98 scrapie was implemented into the national surveillance programmes throughout the world, several cases all over the world was discovered, even in Australia and on New Zealand, countries which has been completely free from scrapie (Cook et al., 2016).

Clinical signs most commonly seen in cases of atypical scrapie are ataxia, nervous behaviour and loss of body condition. Pruritus and the typical scratching behaviour seen in classical scrapie are rarely seen in this type. Using IHC, the PrP^{Sc} deposition in Nor98 appear as fine punctate to coarse granular deposits seen in both grey and white matter. There has never been

reported any intraneuronal staining. Important is also the plaque-like dense focal or multifocal aggregates present in the tectum and in the lateral geniculate body of the midbrain, and the white matter tracts of the cerebral cortex.

After PK treatment, Nor98 scrapie isolates was visualised on a gel as a distinct multiple band pattern, with a rapid migrating band reported to be 11-12 kDa or 7-8 kDa in size (Benestad et al., 2008).

Atypical scrapie has also been detected in goats in several European countries, with similar course of disease, but with far less prevalence than in sheep. Although these numbers might be because of unsatisfactory passive surveillance programmes throughout the world (Curcio et al., 2016).

3.6. Scrapie in Norway

Scrapie was for the first time diagnosed in Norway in 1958 in two rams imported from England. In Norwegian bred sheep it was for the first time suspected in 1981, but the diagnosis was not verified before 1983, after probing on goats. There are, however, several historical descriptions of disease in sheep where the clinical signs have been similar to scrapie as known today. In the following decade after its discovery in the early 80s, scrapie was discovered in a few more flocks, but the outbreaks were only confined to two counties in western Norway (marked as 2 and 3 on figure 4). During the 90s an increasing prevalence of scrapie was registered, with a peak in infected herds in 1995 and 1996 where more than 30 flocks were infected. The epidemic in the mid 90s was for the most constricted to the southern part of Hordaland county and the northern part of Rogaland, but nationwide 6 counties had outbreaks of scrapie. Epidemiological investigations performed later have uncovered that the majority of the infected stocks had been in contact directly or indirectly through trade, shared pastures and

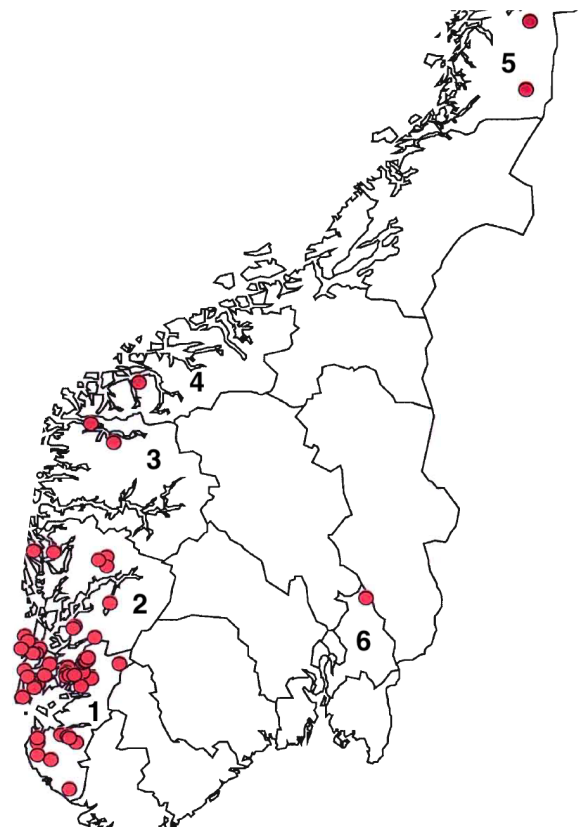


Figure 4: Distribution of Scrapie infected flocks in Norway 1980-2010. Marked counties are: 1: Rogaland; 2: Hordaland; 3: Sogn & Fjordane; 4: Møre & Romsdal; 5: Nordland; 6: Akershus (Hopp et al., 2000).

workers and/or breeding cooperation. However, it was not possible to connect the outbreaks in the other counties Sogn and Fjordane, Møre and Romsdal, Nordland and Akershus to other stocks with scrapie.

A survey of the genotype of the sheep infected with scrapie revealed that it was the cheviot breed that dominated among the PrP^{Sc} infected sheep. A theory was that the disease was imported with this breed before the second world war, giving the disease time to spread over the country. As clinical diagnosis is hard to set, and Norwegian farmers might not have been familiar with the disease and its signs, several years went by before its discovery (Hopp et al., 2000).

The last reported outbreak of classical scrapie in Norwegian sheep was in 2009, where a Texel sheep of genotype ARH/VRQ in Rogaland county in western Norway had been sent to the NVI for necropsy after it had died suddenly. Once the definite diagnosis of infective prions in the diseased sheep was confirmed, the whole stock was killed. Out of more than 500 sheep euthanized as a consequence of scrapie eradication, only four more tested positive for classical scrapie in the flock (Benestad et al., 2010).

After the discovery of the atypical scrapie in 98, several cases have been diagnosed all over the country with the latest cases in 2018 where it was diagnosed in 8 sheep originating from 7 different stocks in 3 different counties (Sviland et al., 2019).

Only one goat herd has tested positive for scrapie in the country. The atypical Nor98 type was demonstrated in 2006 in a 4 years old goat from a fallen stock in Tromsø in the northern part of Norway. None of the goat's 48 flock mates were found positive for Nor98 (Sviland et al., 2007).

3.7. Response from the authorities

In the period from the first outbreak in 1981 and till the last confirmed case in 2009, 59 stocks, distributed over 6 counties (figure 4) had confirmed cases of classical scrapie (Hopp et al., 2000).

After the atypical scrapie, Nor98, was discovered, there are annually diagnosed 5-12 cases in stocks all over the country (Veterinærinstituttet (Norwegian Veterinary Institute), 2018).

After the epidemic in 95/96, there was a drastic decrease of classical scrapie outbreaks, this was mainly because the Norwegian authorities launched a surveillance and control programme for scrapie in 1997. This programme included an information campaign intended for Norwegian sheep farmers, a surveillance programme, control measures in the flocks where scrapie-positive animals have been detected, and measures at the national level restricting the movement of sheep (Hopp et al., 2007).

National guidelines for the management of scrapie were implemented into the eradication programme, as the Norwegian government are obliged to follow international commitments in the battle against scrapie. The Food Safety Authorities' national objective were to eradicate the disease when it was confirmed in a stock with "Stamping out" and with extensive sanitation measures, instead of developing a comprehensive breeding programme.

If classical scrapie is detected in a herd, the herd must be quarantined acc. Regulation on combating animal diseases. All sheep above 12 months euthanized have to be genotyped and tested. All small ruminants, including their breeding products in the primary herd must be euthanised and destructed. In addition, the parents, embryos and eggs to the positive animals, every offspring and siblings to positive ewes and the first offspring to positive rams sold out of the herd must be culled. The building masses storing animals at the farm with a confirmed outbreak of scrapie must be thoroughly sanitised. In some cases, entire stalls and other fixtures must be removed and in worst case scenarios entire barns must be torn down and the ground redeveloped. Contact herds must be traced, and measures taken with these, according to way and extent of contact. Every stall where small ruminants have been present must be held empty for 3 years after the sanitation plan has been executed. When new animals are to be introduced after the quarantine period, the breeding rams to be used must be of the genotype ARR/ARR and ewes with a minimum of one ARR-allele and without VRQ-allele (Mattilsynet, 2006).

As a direct consequence of the scrapie outbreak in the 90s, movement restrictions and regulations for movement and trade of small ruminants between herds were introduced. The country is divided into 4 "Small ruminant regions" geographically, where it is forbidden to move ovine and caprine species across the borders. It is also forbidden to move female animals of these species across county borders, and there was also before 1st of July 2018 forbidden to move male animals across these borders without special permission from the Norwegian FSA. The counties where scrapie was confirmed during the 90s are subject to special restrictions, and there is not allowed to move live animals of sheep out of these counties (Fjermestad-Eie, 2018).

Norwegian small-ruminant herds are also classified into 5 different TSE classes, which are classified on the basis on the duration of the herd's scrapie surveillance:

Class 1: the herd has not been monitored for TSE or has been monitored for a shorter period (<2 years) without TSE, except the atypical Nor98, confirmed.

Class 2: the herd has been monitored for TSE for more than 2 years without a TSE, except the atypical Nor98, confirmed.

Class 3: the herd has been monitored for TSE for more than 3 years without a TSE, except the atypical Nor98, confirmed.

Class 4: the herd meets the conditions set for having a controllable risk of having classical scrapie in section 2 of the TSE regulation §2, cf. Regulation (EC) No 999/2001, Annex VIII, Chapter A, Part A. The animal keeper must apply to the FSA to be in this class.

Class 5: the herd meets the conditions set for having a negligible risk of having classical scrapie in section 2 of the TSE regulation §2, cf. Regulation (EC) No 999/2001, Annex VIII, Chapter A, Part A. The animal keeper must apply to the FSA to be in this class.

The 4th and 5th classes are only relevant for farmers with an aspiration to export to the EU. To be able to trade within the small-ruminant regions, the herd must be in class 3 (Mattilsynet, 2018b).

In addition, a Norwegian case-control-study was started the same year to identify risk factors associated with scrapie. The study was performed as a survey where all stocks with scrapie in 1995, 1996 and first half of 1997 was asked to participate, together with three times as many without detected scrapie in the same veterinary districts (controls). Factors such as transfer of animals between herds, because of for example trade, or contact between animals from different herds, in breeding- or grazing area cooperation were associated with scrapie. In addition, sharing of means of transport, or other equipment and the occurrence of the local sheep breed "Rygja" in the herd were linked to increased risk of scrapie. All these factors were building up to an assumption that contact between animals were important in the transmission of scrapie. Further, the importance of the breeding and breeding cooperation may represent a risk, both because of the direct transmission of infective agents and the transmission of genes more susceptible to the disease. None of the farmers reported that the transmission could be related to vectors, or that the Norwegian feed were contaminated with scrapie prions in meat bone meal (Hopp et al., 2001, 2000).

A questionnaire surveying Norwegian farmers' vigilance in reporting sheep showing scrapie-associated signs was sent to 3000 farmers with sheep in 2002. This study revealed that even though a potential outbreak of scrapie in a herd possibly could lead to the complete destruction of the concerned flock, 97% of the participating farmers stated their willingness to report any suspicion of scrapie in their flocks. The most important condition for this was the Norwegian government's promise to cover all costs regarding the control programme and in case of the need to de-populate a scrapie-positive flock. In Norway there is a standard economical compensation for each animal destroyed due to disease control offered, in addition are the sanitary measures applied fully covered and production subsidies are given during the period where the farm must be empty for up to 3 years (Hopp et al., 2007).

4. Discussion

All prion diseases are similar in that the infective agent are misfolded proteins accumulating in the brain. They all give signs consistent with neuronal damage and will eventually lead to death. Prion diseases are transmissible, but do not necessarily transmit horizontally. Their incubation period is long, although the duration of disease after the onset of clinical signs are rather short. Handling and control of the disease is difficult, as the infective prions residing in the environment is highly resistant to detergents, disinfectants and other sanitising methods. Longer periods of quarantine in areas where prion diseases have been confirmed are the most common way to handle the disease. Animals are often culled, and restrictions are laid on areas and animal stocks. Diagnostics of TSEs is harder than in other infectious diseases, as there is no immunologic response or any antibody/antigen production, which can be detected in blood, lymph or other tissues typical for diagnostic testing. Characteristic histological changes are the cytoplasmic vacuolisation, giving a spongiform appearance of the sampled brain tissues.

Chronic wasting disease and scrapie are similar in many ways. Primarily, these are both diseases caused by infective prions, leading to nervous and behavioural disturbances. Their classical forms are both highly contagious, where infective prions are shed with parturient fluids, saliva, urine and faecal materials. As both reindeer and sheep are social animals, living in herds, bodily fluids are often mixed and the disease can spread rapidly between animals, leading to major outbreaks in short time. Rapid and accurate diagnosis are important when suspicion of a TSE occurs, and as these are notifiable diseases, standardised analytical methods are set. The diagnostic methods are immunohistochemistry, western blot and various rapid tests based on WB- and ELISA-methods, using tissue from the obex-region in the brain, meaning that samples only are collectable post-mortem. Both CWD and scrapie prions can be found in the LRS tissues, and methods using RAMALT or nasal swabs of the olfactory mucosa are under development for perimortem diagnostics.

Scrapie and CWD are also similar in that there have been described atypical forms of the prion diseases in both. These are TSEs in that they are caused by misfolded prion proteins and are to an extent transmissible, but studies and observations done have revealed that they most likely not are as contagious as the classical forms. These are supported by their sporadic appearance geographically, the fact that they are found almost exclusively in elder animals and the complete absence of prions in lymph nodes.

When classical scrapie is detected in a herd, stamping out is necessary by law. Such drastic measures in sheep are practically easy to perform, as domestic small ruminants most often are confined in buildings or are grazing within fenced areas. Further restrictions and contact investigations are initiated, an empty period for at least 3 years and thorough sanitation measures must be performed in the buildings housing infected animals. The economic aspects with these measures are for the most covered by the Norwegian government and insurances, making sure the financial losses are at a minimum. Contact stocks must be traced, and measures taken according to degree of contact with the primary herd. As movement and trade of sheep have to be authorized by the FSA, and later reported to given registers, in addition to all stocks grazing in the same areas in the summer also must be registered and easy to trace, the work in determining contact herds should be rather uncomplicated. An increased control of all small-ruminant herds in the area and an enhanced sampling frequency must be introduced to all slaughterhouses. The official national free status will also be lost, or the process in getting a free status will be nullified, in case of a new epizootic, having an impact on the country's meat export and trade. The affected herd will also be placed to the lowest TSE class. According to the survey performed in Norwegian sheep farmers in 2002, farmers are willing to cooperate with the reporting of scrapie suspicion and seem to have an awareness of the possible consequences in not reporting such infectious diseases. The movement of sheep across county borders are heavily restricted, and permission can be granted by the Norwegian food safety authorities only in special cases.

As scrapie has been a familiar disease in Norway for almost 40 years, procedures on measures to be taken in case of outbreaks are well-established and responsible authorities, veterinarians and farmers have an awareness and knowledge on why such measures must be performed to protect the sheep welfare and industry in the country.

As for the CWD outbreak in wild reindeer in Nordfjella, no such regulations and procedures were accessible for how to handle the disease to avoid spread to other cervids and potential major animal losses. The competent authorities had to seek out advice from those handling the cervid prion disease in northern-America and try to apply those to the Norwegian situation. As the disease was found in only one herd, which was relatively isolated from other herds because of the highway dividing the area into two zones, the decision to stamp out this entire herd and follow through a fallow period on the basis of such few confirmed cases was easier than if more herds and cervid populations had been involved. It is harder to justify such

measures to the common folks when it is a rare and unknown disease, and it previously has been emphasized that the Norwegian reindeer is highly valuable, and the population must be managed carefully. Therefore, it was important to proceed information campaigns about the disease and to involve the locals in the area when the culling strategy was decided. There was some hesitation and resistance against the reindeer cull, but most of the locals participating in hunting understood the necessity of the measures, eventually. As tracking down contact animals and potential contagious carriers of the disease were impossible, as wild cervids may wander great distances, and their patterns may vary for each time they are relocating, it was decided to increase the ordinary hunting quotas, especially in the areas surrounding Nordfjella. This was to closely investigate whether there still were reindeer infected with CWD alive, in addition to decrease the density of the cervid population. After the fallow period, reintroduction of healthy and resistant animals is important. As there are studies on cervid's genetic susceptibility to prion diseases ongoing, and with a view especially on reindeer, these can provide important information for the reintroduction. If these research projects are concluded in time, they can provide important information on which genetic lines that might be naturally resistant to CWD and therefore will have an advantage living in Nordfjella zone 1. If there is found a genetic resistance to CWD in some reindeer, it would be like the restrictions laid on the reintroduction of sheep into farms that have been infected with scrapie.

Cooperation was also important with the sheep farmers having their sheep grazing in the Nordfjella zones during the summer and had licking stone areas spread for their sheep, since these were considered huge risk factors for transmission of prions. The removal of these mineral stones could lead to the sheep flocks getting more spread in the regions where they were grazing and making it harder for the farmers to inspect their animals during the summer and collect them and bring them home in autumn. A compromise was therefore at first made between the FSA and farmers to make fences around these stones, making sure only sheep could get through. As it later was revealed, by cameras placed out at these licking stone enclosures, that reindeer indeed was able to reach the licking stones regardless of the fences, it was later decided to completely ban such licking stone areas in the Nordfjella zones. After the reindeer in Nordfjella zone 1 was completely removed, a fallow period, which is currently in process, was deemed necessary. The duration of such a quarantine period was, due to lack of sufficient data, hard to decide. A review on the current records on infective prions' resistance and capability to remain infective in the environment was performed, and for time being, the quarantine is set to a minimum of 5 years before reintroduction of healthy reindeer.

A goal to reduce the density of the cervid populations in southern Norway was also set, as continuous high counts of deer-species in these areas might lead to an increased risk of CWD. Consequently, the disease could spread to other areas, cervid species and eventually to the Sámi-peoples' semi-domesticated reindeer herds further north, which also have grazing areas across the Swedish and Finnish borders, potentially spreading the disease to deer-populations in neighbouring countries.

A massive screening programme was launched following the discovery of CWD in 2016, with today more than 90 000 samples from cervid animals tested, collected by hunters during the hunting season, samples taken at game collection centres, from roadkill or self-dead animals etc.

Because of the Norwegian outbreak, EFSA adopted a three-year surveillance programme for CWD in cervids from 2018 in 6 EU member countries which have a population of reindeer and/or moose. Cases of the atypical CWD have already been confirmed in moose in both Sweden (3 cases) and Finland (1 case), and considering the discovery of the atypical type, which is found in both moose and red deer, all EU countries could benefit being part of such a surveillance programme, as there are major deer animal populations throughout Europe.

4.1. Conclusion

The prion diseases scrapie and CWD were both handled by stamping-out, and restrictions laid on the housing or areas the infected animals were residing, laying a quarantine period on the holdings for 3-5 years. For the current situation, this seem to have been the best resolutions for both diseases, as there for scrapie only have been few outbreaks of the classical type since the onset of the surveillance programme. Also, for the classical reindeer CWD, no further cases have been discovered since the cull in 2017-2018. Although, it is still early to conclude, as screening are still ongoing, and the quarantined zone still is empty.

Justification for the cull, however, was easier to get through to the people regarding the scrapie-outbreak, as there was much more resistance when the reindeer were to be culled due to a similar disease. In addition, the ban on licking stones spread out in the environment in the Nordfjella zones did initiate a conflict with the sheep farmers in the area.

5. Summary

Prion diseases are a family of rare and fatal progressive neurodegenerative diseases, characterised by incubation periods up to several months or years, spongiform changes seen as neuronal loss, and no inflammatory response, commonly seen in more frequent viral and bacterial diseases, are visible in blood or cerebrospinal fluid

The most known prion diseases are chronic wasting disease (CWD) in cervids, scrapie in sheep and goat, and bovine spongiform encephalopathy (BSE) in cattle. Creutzfeldt-Jakob disease (CJD) and the variant form (vCJD) and Kuru are examples of prion diseases occurring in humans.

CWD is a fatal, neurodegenerative prion disease diagnosed in the family of Cervidae, including captive and free-ranging deer, elk, moose and reindeer in North America, South Korea, and recently in Scandinavia.

CWD is one of the few TSEs considered highly infectious and are believed to be horizontally transmitted.

In March 2016, CWD was for the first time diagnosed in Europe. A female reindeer from the wild reindeer area called “Nordfjella region zone 1” died during an attempt to chemically immobilise wild reindeer in Norway. In May and June, the same year, CWD was diagnosed in two Eurasian moose approx. 300 km. north of the first confirmed case, in Trøndelag county in central Norway and was for the first time discovered in red deer in Norway, October 2017 in the municipality of Gjemnes in mid-western Norway.

Classical scrapie is a fatal, neurodegenerative prion disease affecting ovine and caprine species. The disease is known for almost 300 years, as the first case reported was in Great Britain in 1732 in sheep. Scrapie was for the first time diagnosed in Norway in 1958 in two rams imported from England, and Norwegian bred sheep in 1981.

The prion diseases scrapie and CWD were both handled by stamping-out, and restrictions laid on the housing or areas the infected animals were residing, laying a quarantine period on the holdings for 3-5 years. For the current situation, this seem to have been the best resolutions for both diseases, as there for scrapie only have been few outbreaks of the classical type since the onset of the surveillance programme. Also, for the classical reindeer CWD, no further cases have been discovered since the cull in 2017-2018. Although, it is still early to conclude, as screening are still ongoing, and the quarantined zone still is empty.

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7. Appendices

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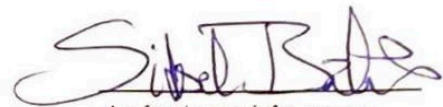


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Based on the above, HuVetA aims to:

- *increase awareness of Hungarian veterinary science not only in Hungary, but also internationally;*
- *increase citation numbers of publications authored by Hungarian veterinarians, thus improve the impact factor of Hungarian veterinary journals;*
- *present the knowledge base of the University of Veterinary Medicine Budapest and its partners in a focussed way in order to improve the prestige of the Hungarian veterinary profession, and the competitiveness of the organizations in question;*
- *facilitate professional relations and collaboration;*
- *support open access.*

7.2. Supervisor counter-signature form.

Appendix 4. Supervisor counter-signature form

I hereby confirm that I am familiar with the content of the thesis entitled
Chronic Wasting Disease Outbreak in Scandinavia

.....
written by Sidsel Trygstad Bakås (student name)

which I deem suitable for submission and defence.

Date: Budapest, 20.....day 11.....month 2019.....year


.....
Prof. Tamás Bakonyi
Supervisor name and signature

.....
Department of Microbiology and Infectious Diseases

.....
Department