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## First Detection of Chronic Wasting Disease in a Wild Red Deer (*Cervus elaphus*) in Europe

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**ABSTRACT:** Chronic wasting disease (CWD) is a fatal contagious prion disease naturally occurring in cervids in North America. In 2016, CWD was detected in wild reindeer (*Rangifer tarandus*) and moose (*Alces alces*) in Norway. Here, we report the first known naturally infected wild Norwegian red deer (*Cervus elaphus*).

Chronic wasting disease (CWD) is a fatal neurodegenerative disorder that affects wild and farmed cervids. It is a prion disease belonging to the transmissible spongiform encephalopathies. The main distribution is North America, and natural infections occur in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and less frequently in moose (*Alces alces*; Williams 2005; Baeten et al. 2007). Recently, CWD was detected in Europe in wild reindeer (*Rangifer tarandus*) (Benestad et al. 2016) and moose (Pirisinu et al. 2018) from Norway and in one moose from Finland (Finnish Food Safety Authority Evira 2018). In captive red deer (*Cervus elaphus*), natural CWD has been reported in a single animal in Minnesota, US (Schwablander et al. 2013) and recently in a farm in Quebec, Canada (Canadian Food Inspection Agency 2018), respectively. Experimentally, CWD has been transmitted to various cervids, including red deer (Balachandran et al. 2010; Haley and Hoover 2015).

Norway has an estimated winter population of 335,000 wild red deer, moose, roe deer (*Capreolus capreolus*), and reindeer, in total (Austrheim et al. 2011), and approximately 120 deer farms, keeping mainly red deer and some fallow deer (*Dama dama*). The red deer inhabit southern Norway up to 66°N, with the

main population along the west coast, and 42,541 were harvested by hunters in 2017 (Statistics Norway 2018). Here, we report the first case of CWD in a wild red deer worldwide.

An adult female red deer was shot by a hunter in October 2017 in Gjemnes municipality in western Norway (Fig. 1). Before being shot, she was grazing alone on farmland with no signs of disease. She was field dressed, and the head and carcass were delivered to a game slaughterhouse. A spoon sample of the medulla oblongata (MO) was submitted to the Norwegian Veterinary Institute (Oslo, Norway) as part of a national CWD surveillance program. After the initial positive CWD result by routine TeSeE Short Assay Protocol (SAP) enzyme-linked immunosorbent assay (ELISA) (Bio-Rad, Hercules, California, USA) for detection of abnormal prion protein (PrP<sup>res</sup>), the available remains of the animal were examined at Norwegian Veterinary Institute.

The deer was estimated to be 16 yr old based on cementum annuli in the first incisor root (Hamlin et al. 2000). Necropsy of the head, carcass, and hide revealed good body condition and no gross pathologic lesions. Half of the brain tissue remaining after the spoon sample, half of various lymph nodes (LN), and the tonsils were frozen, and the other halves were fixed in 10% buffered formalin. Samples of brain, LN, and tonsil were processed routinely for immunohistochemical and histologic examination.

The MO and five LNs (retropharyngeal, mandibular, parotid, prescapular, popliteal) were analyzed using TeSeE SAP ELISA. Both the initial sample and second sample of MO at



FIGURE 1. Map of Norway showing the location of wild cervids found positive for chronic wasting disease (CWD) in the period from 2016 through 29 October 2018. The CWD-positive red deer (*Cervus elaphus*) was in Gjernes municipality (blue filled circle). The CWD-positive moose (*Alces alces*) were in the municipalities of Selbu, 2016 ( $n=2$ ) and Lierne, 2017 ( $n=1$ ; green filled circles). The Nordfjella reindeer area is functionally divided into two zones, and all ( $n=19$ ) the CWD-positive wild reindeer (*Rangifer tarandus*) were found in the northern zone, referred to as zone 1 (red).

the obex area were positive (optical density values 3.5 and 0.330), whereas all LNs were negative. The presence of CWD also was confirmed by the detection of  $\text{PrP}^{\text{res}}$  in MO by using the TeSeE western Blot test (Bio-Rad). Histopathologic examination of MO was inconclusive for spongiform degeneration due to autolysis. No intraneuronal vacuolation was observed.

Sections of MO, LNs, and tonsil were prepared for immunohistochemical examination to demonstrate accumulation of  $\text{PrP}^{\text{res}}$  as in Benestad et al. (2016) using the antibodies mAb L42, F99/97.6.1, and 12B2. In MO, F99/97.6.1 and L42 gave similar staining, with

coarse granular deposits in the neuropil, and perineuronal, intraneuronal, and linear staining in several nuclei and axonal tracts. The immunostaining obtained with 12B2 was weaker and gave no intraneuronal staining. The LNs and tonsils showed no  $\text{PrP}^{\text{res}}$  staining. Genomic DNA was extracted from brain tissue, and the prion protein gene open reading frame was amplified and sequenced (Applied Biosystems 3500XL genetic analyzer with Big Dye Terminator chemistry, Foster City, California, USA), showing glutamic acid (E) homozygosity in codon 226.

Based on brain tissue being found positive for  $\text{PrP}^{\text{res}}$  in three diagnostic tests, we concluded that this represented the first detection of CWD in wild red deer. Elk and red deer are closely related species (*Cervus* spp.). Experimentally, red deer were found to be susceptible to CWD after oral inoculation of prions from elk (Balachandran et al. 2010), accumulating  $\text{PrP}^{\text{res}}$  in brain, lymphoreticular, and other peripheral tissues. Natural CWD was reported in a young captive red deer from Minnesota having  $\text{PrP}^{\text{res}}$  positive brain tissue and LNs (Schwablander et al. 2013). This contrasts with our case, where no detectable  $\text{PrP}^{\text{res}}$  was found in the LNs and tonsils. However, some elk infected with CWD show deposits of  $\text{PrP}^{\text{res}}$  only in the brainstem and not in lymphoid tissues (Spraker et al. 2004, 2015).

Comprehensive surveillance was implemented after detecting CWD in Norway in 2016. Of 59,533 cervids tested from 2016 to 29 October 2018, CWD was diagnosed in 19 reindeer (all from Nordfjella mountain area), three moose (two from Selbu municipality, one from Lierne municipality), and the herein reported red deer from Gjernes municipality (Fig. 1). In total, 11,398 wild red deer were tested, including 236 from Gjernes (Norwegian Veterinary Institute 2018).

All of Norway is populated by one or more cervid species with varying degrees of overlapping distribution range. A relevant question is whether there is any migration of cervids between the CWD-positive populations. Gjernes is located about 200 km north of Nordfjella, 170 km west of Selbu, and 335

km southwest of Lierne (Fig. 1). Seasonal migration is common, and distances may exceed 150 km, but migration distances for red deer in western Norway are usually in the range of 20–40 km (Meisingset et al. 2018).

The origin of CWD in Norwegian wild cervids is unknown. A major question is whether the CWD detected in reindeer, moose, and red deer represents different CWD strains. The CWD from moose in Norway was characterized as an atypical CWD identified as Nor16CWD (Pirisinu et al. 2018). In this context, to better understand the red deer isolate, further molecular analysis and bioassays are currently being done. The prevalence, epidemiology, and implications of the type of CWD discovered in this red deer remain to be determined.

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