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A Natural Case of Chronic Wasting Disease in a Free-ranging Moose (*Alces alces shirasi*)

Laurie A. Baeten,[1,4] Barbara E. Powers,[2] Jean E. Jewell,[3] Terry R. Spraker,[2] and Michael W. Miller[1]
[1] Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526-2097, USA; [2] Colorado State University, Veterinary Diagnostic Laboratory, Fort Collins, Colorado 80523, USA; [3] Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming 82070, USA; [4] Corresponding author (email: laurie.baeten@state.co.us)

ABSTRACT: Chronic wasting disease (CWD) was diagnosed in a free-ranging moose (*Alces alces shirasi*) killed by a hunter in Jackson County, Colorado, USA, in September 2005. The diagnosis was based upon immunohistochemistry (IHC) demonstrating the presence of accumulations of CWD-associated prion protein (PrP^{CWD}) in tissue sections of medulla oblongata at the level of the obex (dorsal motor nucleus of the vagus) and in retropharyngeal lymph node (RPLN); additional testing by IHC revealed deposits of PrP^{CWD} in multiple sections of medulla oblongata and cervical spinal cord as well as palatine tonsil and submandibular lymph node tissues. Western blot confirmed the presence of PrP^{CWD} in RPLN and tonsil tissue. The PrP^{CWD} also was detected via enzyme-linked immunosorbent assay of RPLN tissue. Spongiform encephalopathy was observed in sections of the brainstem and cervical spinal cord, although no clinical signs were noted by the hunter who killed the animal. The affected moose was homozygous for methionine at codon 209 of the prion protein coding region. In October 2006, two additional free-ranging moose were diagnosed with CWD. Epidemiology and implications of CWD in moose remain to be determined.

Key words: *Alces alces shirasi*, chronic wasting disease, immunohistochemistry, moose, prion, PrP^{CWD}, Western blot.

Chronic wasting disease (CWD) (Williams and Young, 1980) is an endemic prion disease of free-ranging deer (*Odocoileus* spp.) and wapiti (*Cervus elaphus nelsoni*) in north central Colorado, USA, and southeastern Wyoming, USA (Williams and Young, 1992; Spraker et al., 1997; Miller et al., 2000). Chronic wasting disease occurs naturally in both deer and wapiti, but it has not been reported to occur naturally in other North American cervid species. Because of their close taxonomic relationship and similarities in DNA sequences of the prion protein (PrP) coding region to deer and wapiti, it had been hypothesized that moose (*Alces alces shirasi*) would be naturally susceptible to infection if sufficient exposure to the CWD agent occurred (Williams, 2005). A recent experiment using oral exposure to infectious brain tissue in captive moose confirmed that this species is susceptible to CWD (Kreeger et al., 2006). Here, we report a natural case of CWD in a free-ranging moose from north central Colorado.

Historically, moose were rare in Colorado (Bailey, 1944) and absent for decades until reintroduced into the Illinois River drainage in North Park (Jackson County) in north central Colorado in the late 1970s (Duvall and Schoonveld, 1988). The original source stock was from northwestern Wyoming and northeastern Utah. The initial restoration effort was supplemented by releases in the Laramie River drainage in northwestern Larimer County and more recently near Creede and on the Grand Mesa. In 2004, the Colorado Division of Wildlife (CDOW) estimated that about 1,300 moose resided in Colorado, mostly in the north central part of the state near the original transplant area (CDOW, unpubl. data).

Moose in Colorado have been examined for evidence of health problems, including CWD infection since the early 1990s. None of 41 sick or dead moose submitted from throughout Colorado for diagnostic evaluation showed any neurological lesions or disease-associated PrP (PrP^{CWD}) deposition consistent with CWD infection (CDOW, unpubl. data), and none of 228 moose submitted through harvest surveys conducted by the CDOW during 2002–

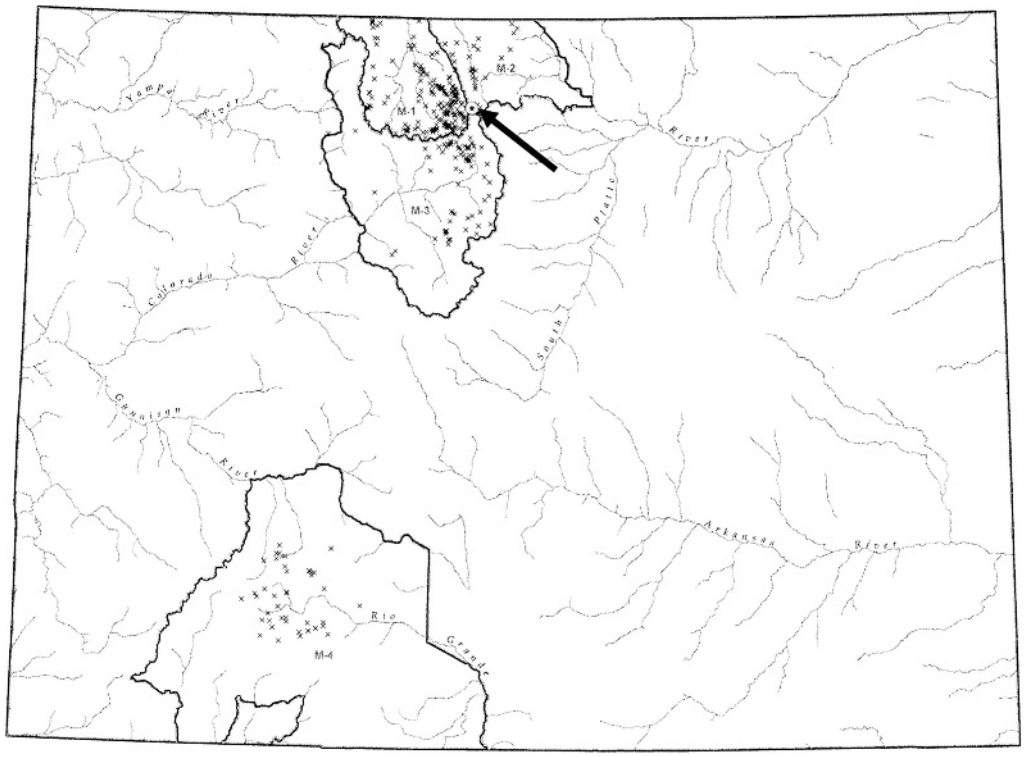

FIGURE 1. Geographic distribution of samples from moose collected for chronic wasting disease (CWD) surveillance in Colorado, USA, 2002–2005, and location of harvested moose infected with CWD (highlighted by arrow). M1=218; M2=29; M3=84; M4=49.

2004 showed evidence of CWD infection. In 2005, however, one of 159 harvested moose submitted did show evidence of CWD infection (Fig. 1). The infected moose was an adult (>2-yr-old) male shot by an archer on 10 September 2005 in Jackson county, Colorado (Fig. 1). In interviews after laboratory findings were revealed, the archer did not recall seeing clinical signs consistent with those described for CWD-infected deer and wapiti in the harvested moose or other moose seen in the vicinity of the harvest location. The archer indicated that behavior of the infected moose was the same as observed in other male moose in previous hunts. Based on photographs and observations made by the hunter during field dressing of the carcass, the infected moose apparently was in good body condition with normal amounts of fat. No unusual appearances of the carcass or visceral organs were reported by the hunter.

The head from this moose was submitted to the CDOW in compliance with a mandatory statewide CWD surveillance program for harvested moose. Samples of caudal brainstem including medulla oblongata as well as the palatine tonsils, retropharyngeal lymph nodes (RPLNs), and submandibular lymph nodes (SMLNs) were dissected from the head. The entire brainstem sample, along with one RPLN, was fixed in 10% neutral buffered formalin. Palatine tonsils, SMLNs, and the other RPLNs were stored frozen at −20 C; subsamples of tonsil and SMLNs were subsequently fixed in 10% neutral buffered formalin.

Fixed tissues were submitted to the Colorado State University Veterinary Diagnostic Laboratory (Fort Collins, Colora-

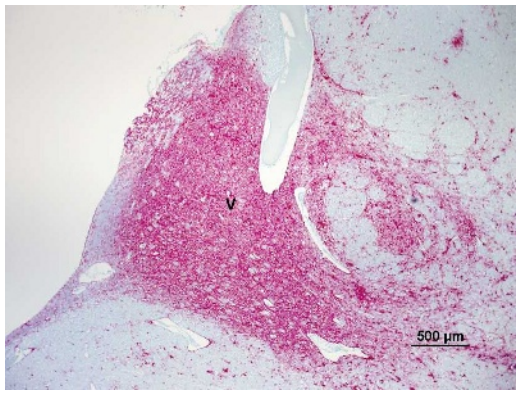

FIGURE 2. Immunohistochemistry demonstrated tissue deposits of chronic wasting disease-associated prion protein (PrP^{CWD}). PrP^{CWD}-associated chromagen deposits (fuchsia) were present bilaterally throughout the dorsal motor nuclei of the vagus nerve (V).

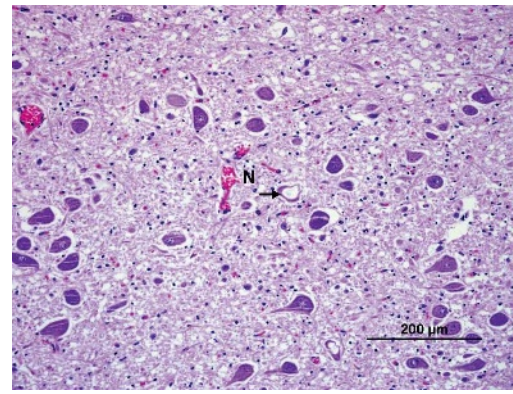

FIGURE 3. Photomicrograph of the dorsal motor nucleus of the vagus nerve of the brain stem of a moose with chronic wasting disease. Note the vacuoles within the neuropil (arrows) and the vacuole within the cytoplasm of a neuron (N). H&E stain. Bar=200 µm.

do, USA) for microscopic evaluation. Sections of medulla oblongata at the level of the obex and RPLN cortex were prepared for immunohistochemistry (IHC) by using monoclonal antibody F99/97.6.1 (O'Rourke et al., 2000) and techniques described by Spraker et al. (2002a, b); subsequently, sections of palatine tonsil, SMLNs, and brainstem at the levels of the gracilis ganglion and first cervical segment of the spinal cord were prepared for IHC by using the same methods. Initial examination revealed PrP^{CWD}-associated chromagen deposits in germinal centers of numerous lymphoid follicles of the RPLNs as well as bilaterally throughout the dorsal motor nuclei of the vagus nerve (DMNV) (Fig. 2); in the DMNV, chromagen deposits were primarily associated with astrocytes in the neuropil, although some intracytoplasmic deposits in neurons were observed. Patterns of chromagen deposition in palatine tonsil and SMLNs were essentially the same as seen in RPLNs. In addition to the DMNV, IHC revealed chromagen deposits indicative of PrP^{CWD} accumulation in the solitarius, cuneate, reticular, and hypoglossal nuclei as well as the nucleus ambiguus and the nucleus of the spinal tract of the trigeminal nerve. With the exception of intraneuronal chromagen deposition being somewhat more common in this case, the patterns observed were virtually indistinguishable from those described previously in cases of CWD in deer and wapiti (Spraker et al., 1997, 2002a, b; Miller and Williams, 2002; Williams, 2005) and in a moose experimentally infected with CWD (Kreeger et al., 2006).

Three sections of brainstem at the levels of the vagus nucleus, gracilis ganglion and anterior first cervical segment of spinal cord were stained with hematoxylin and eosin (H&E) for histologic evaluation. Sections of the DMNV contained four neurons with vacuoles of various sizes (three vacuoles on one side, one vacuole on the other side) (Fig. 3). No vacuolated neurons were noted in the solitarius, cuneate, reticular, olivary, or hypoglossal nuclei or in the nucleus ambiguous, mid raphe, and the nucleus of the spinal tract of the trigeminal nerve. However, mild-to-moderate spongy degeneration was noted in the neuropil of all of the aforementioned sites. The mid raphe also showed vacuolization in the white matter. These spongiform changes were typical of lesions seen in deer and wapiti with CWD (Williams and Young, 1993; Spraker et al., 1997, 2002a, b; Williams, 2005).

Fresh palatine tonsil and RPLN extracts were analyzed by Western immunoblot (WB) by using established methods (Kreeger et al., 2006) as an independent assessment of CWD infection. The WB results confirmed presence of protease K-resistant PrP in both RPLNs and tonsil of the harvested moose, and the blot produced a PrP^{CWD} banding pattern that was essentially the same as banding patterns seen in brain and lymphoid tissues from experimentally infected moose or wapiti.

To assess the potential reliability of screening tests used routinely in CWD surveillance for detecting cases in moose, we submitted fresh RPLN for enzyme-linked immunosorbent assay (ELISA). Tissue was prepared and analyzed using methods described previously (Hibler et al., 2003). On the basis of criteria developed for deer and wapiti (Hibler et al., 2003), an optical density (OD) value of 0.10 was regarded as the cutoff for a suspect reaction. The OD value from analysis of RPLNs from the infected moose was 0.94, indicating that this ELISA has the ability to detect CWD in moose and possibly other susceptible cervid species.

Fresh RPLNs were prepared to extract DNA (DNeasy tissue kit, QIAGEN, Valencia, California, USA) and submitted to the Wyoming Game and Fish Department's Forensic Laboratory (Laramie, Wyoming, USA) for analysis of mitochondrial DNA. Using methods described by Murray et al. (1995), DNA from the RPLN sample was confirmed as having originated from a moose. Separately, genomic DNA also was extracted from frozen RPLN tissues, and the PrP gene coding region was sequenced as described previously for mule deer (*Odocoileus hemionus*) (Jewell et al., 2005). The DNA sequence of the PrP coding region was homozygous for methionine at codon 209, and it did not carry the isoleucine polymorphism frequently found at that position in this species (Kreeger et al., 2006; also see GenBank accession nos. AY225484 [209M] and AY225485 [209I]).

The source of infection in this moose could not be determined, but it seems most likely to be from direct or indirect exposure (Miller et al., 2004) to sympatric mule deer or wapiti infected with CWD. Home ranges of moose in north central Colorado may seasonally overlap to some extent with the ranges of deer and wapiti populations where CWD is endemic (Miller et al., 2000; Colorado Division of Wildlife, 2004; Conner and Miller, 2004). In North Park, adult male moose move between relatively distinct seasonal ranges with overall home ranges (estimated here from median minimum convex polygons) that typically exceed 50 km^2 (Kufeld and Bowden, 1995). Moose in this area tend to select habitats dominated by willow (*Salix* spp.) or lodgepole pine (*Pinus contorta*) (Kufeld and Bowden, 1995), and in western Larimer and eastern Jackson and Grand counties they are commonly encountered in proximity to locations where both mule deer and wapiti are found during the summer (Wolfe, pers. comm.). Assuming "average" behavior and movement for the infected moose, this animal's summer and autumn ranges potentially could have overlapped with summer ranges of mule deer from at least three winter ranges where CWD is well-established (Conner and Miller, 2004) as well as with wapiti from the Poudre River and Estes Park/Rocky Mountain National Park herds where CWD also is endemic (Spraker et al., 1997; Miller et al., 2000). Recent 3-yr (2002–2004) pooled CWD prevalence estimates in the four data analysis units (DAUs) surrounding the harvest location were 0.005–0.076 for deer and 0.003–0.021 for wapiti (CDOW, unpubl. data), suggesting that some form of direct or indirect interaction with an infected deer or wapiti was not improbable. Alternatively, some other source of infection could have given rise to this case.

In October 2006, two additional free-ranging moose were diagnosed with

CWD. Both moose were adult males killed by hunters. The moose were located in the same DAU and an adjacent DAU as the index case. Diagnosis was confirmed by IHC with staining patterns similar to those seen in the other free-ranging and experimental cases. The OD value from ELISA analysis of RPLNs from the infected moose were 1.620 and 1.017. Further evaluation and characterization of these cases are in progress.

The epidemiology and implications of CWD in moose remain to be determined. Based on available surveillance data, the 3-yr (2003–2005) pooled CWD prevalence estimate among moose in DAU M-1 in north central Colorado is 0.005 (1/219; 0.5%) (95% confidence interval 0.0001–0.025); insufficient data are available to assess temporal trends. The mechanism(s) of transmission of CWD within and between susceptible host species is not completely understood, and whether CWD is contagious among moose is not presently known. In light of the similarities in patterns and intensity of PrP^{CWD} deposition in lymphoid tissue between this case and cases in deer and wapiti and the apparent relationship between such patterns and the natural transmission of prion diseases in various ruminant hosts (Kimberlin and Walker, 1989; Sigurdson et al., 1999; Miller and Williams, 2003; Miller and Wild, 2004), transmission among moose should be expected. However, moose in this area typically live alone or in groups of two to five individuals (Kufeld and Bowden, 1995); thus, social behavior and segregation may diminish the likelihood of CWD epidemics occurring in moose in north central Colorado that are comparable in magnitude to those seen in deer or wapiti.

Given our findings, we encourage wildlife managers to include moose in CWD surveillance and to consider CWD in monitoring health problems in moose populations, particularly where those populations share habitat with other cervids that may have been exposed to CWD. Moreover, CWD also should be added to the list of diseases to be considered in translocation efforts to re-establish moose in unoccupied habitats.

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