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SHORT COMMUNICATIONS

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Oral Transmission of Chronic Wasting Disease in Captive Shira's Moose

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ABSTRACT: Three captive Shira's moose (*Alces alces shirasi*) were orally inoculated with a single dose (5 g) of whole-brain homogenate prepared from chronic wasting disease (CWD)–affected mule deer (*Odocoileus hemionus*). All moose died of causes thought to be other than CWD. Histologic examination of one female moose dying 465 days postinoculation revealed spongiform change in the neuropil, typical of transmissible spongiform encephalopathy. Immunohistochemistry staining for the proteinase-resistant isoform of the prion protein was observed in multiple lymphoid and nervous tissues. Western blot and enzyme-linked immunosorbent assays provided additional confirmation of CWD. These results represent the first report of experimental CWD in moose.

Key words: *Alces alces shirasi*, chronic wasting disease, enzyme-linked immunosorbent assay, immunohistochemistry, moose, oral inoculation, prion, PrP^{CWD}.

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy, presumably caused by a proteinase-resistant isoform (PrP^{CWD}) of the prion protein (Williams and Young, 1980). The known natural hosts for CWD are North American cervids: mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*; Williams et al., 2002). It is unknown how PrP^{CWD} is naturally transmitted from an infected host to a susceptible animal; however, experimental transmission has been achieved through oral (Sigurdson et al., 1999) or intracerebral (Williams and Young, 1992) inoculation with brain suspension from CWD-infected mule deer or elk.

The Shira's moose (*Alces alces shirasi*) is another North American cervid, which inhabits the CWD endemic area of northern Colorado and southeastern Wyoming. Because moose could be exposed to CWD from infected elk or deer, there is concern that CWD could eventually become established within moose populations. We report on the experimental oral inoculation of captive Shira's moose with CWD.

This study was conducted at the Wyoming Game and Fish Department's Sybille Wildlife Research Unit (Sybille), Wheatland, Wyoming, USA (41°45.778′N, 105°22.605′W). Orphan moose (4–12 mo old) from northwestern and north-central Wyoming, where CWD has not been documented by surveillance (Kreeger et al., unpubl. data), were opportunistically transported to the Sybille facility.

Moose were inoculated with a single dose (5 g) of whole-brain homogenate prepared from CWD-affected captive mule deer (Sigurdson et al., 1999). The inoculum was prepared by mixing brain homogenate with approximately an equal volume of honey and loaded into syringes modified by cutting off the needle port. Moose were manually restrained, and the brain material was inoculated into the oral cavity. Moose were observed for several minutes post-administration to ensure consumption of the inoculum. The 5 g of brain homogenate corresponded to an estimated 30 μg of disease-associated prion protein (Raymond et al., 2000). The infectivity of this pooled material had previously been ascertained in elk and mule deer, although not quantified (Williams et al., 2002; Kreeger et al., unpubl. data).

One male (moose 1) and female (moose 2) were inoculated in April 2002, and another female (moose 3) was inoculated in January 2003. Once inoculated, moose were then released into an 8.1-ha pasture containing natural forage and water and supplemented with alfalfa hay and a pelleted ration that did not contain ruminant protein. Daily observations of moose were attempted, but this was not always possible because of hilly and wooded terrain that concealed the moose.

Upon death, moose were necropsied and from 35–50 different tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E) for histologic examination. Tissues were also examined for disease-associated prion protein by immunohistochemistry (IHC) as previously described for mule deer and elk (Miller and Williams, 2002), by Western blot as previously described (Jewell et al., 2005), and by enzyme-linked immunosorbent assay (ELISA; TeSeE Purification and Detection, BioRad Laboratories, Hercules, California, USA). In brief, tissue sections for IHC were mounted on coated glass slides and were pretreated in formic acid followed by autoclaving at 121 C in Target Retrieval Solution (DAKO Corp., Carpinteria, California, USA). The primary antibody used in the study was F99/97.6.1 (VMRD Inc., Pullman, Washington, USA) followed by alkaline phosphatase secondary detection and fast red chromagen (Ventana Medical System, Tucson, Arizona, USA). The ELISA was performed on frozen tissues according to the manufacturer's protocols with an optical density cutoff value calculated as specified for cervid retropharyngeal lymph node assays.

All moose died without showing any characteristic clinical signs (Williams and Young, 1992) before death that are indicative of CWD in elk or deer. Moose 1 died 113 days postinoculation, and its tissues were extremely autolytic by the time the carcass was located. However, deposition of PrP^{CWD} in retropharyngeal lymph nodes (RLN) and tonsilar tissues were confirmed by IHC. Analysis by ELISA was not conducted on these samples.

Moose 2 died 567 days postinoculation. Brain lesions compatible with a spongiform encephalopathy were not evident in H&E-stained sections. Immunohistochemical staining for PrP^{CWD} was equivocal. At the level of the obex, minimal bilateral, but unequal in severity, staining was observed in a small area along the ventrolateral aspect of the dorsal motor nucleus of the vagus nerve (DMNV). No staining for PrP^{CWD} was detected in tonsil or lymph nodes of the head and neck. The ELISA assays of tissues from nervous system, lymphoid system, endocrine and salivary glands, viscera, bone marrow, and reproductive organs produced negative results, with the exception of an elevated optical density value below the positive cutoff value for one mesenteric lymph node.

Moose 3 died 465 days postinoculation. Histologic examination revealed spongiform change in the neuropil, typical of the transmissible spongiform encephalopathies (Fig. 1A). This occurred in areas of the brain with the most intense PrP^{CWD} IHC staining, such as the DMNV and supraoptic and paraventricular nuclei of the hypothalamus. Neuronal cytoplasmic spongiform change was evident in only a few neuronal cell bodies in these nuclei. Overt astrogliosis was not appreciated; however, special staining for astrocytes (i.e., glial fibrillary acidic protein IHC) was not done.

Positive IHC staining for PrP^{CWD} was detected in many brain areas. In the medulla oblongata, the most intense PrP^{CWD} IHC staining occurred in the DMNV extending laterally to the nucleus of the tractus solitarius. Staining consisted of dense and often coarse granular deposits of bright red chromagen at the periphery of neuronal cell bodies, in perivascular areas, and throughout the

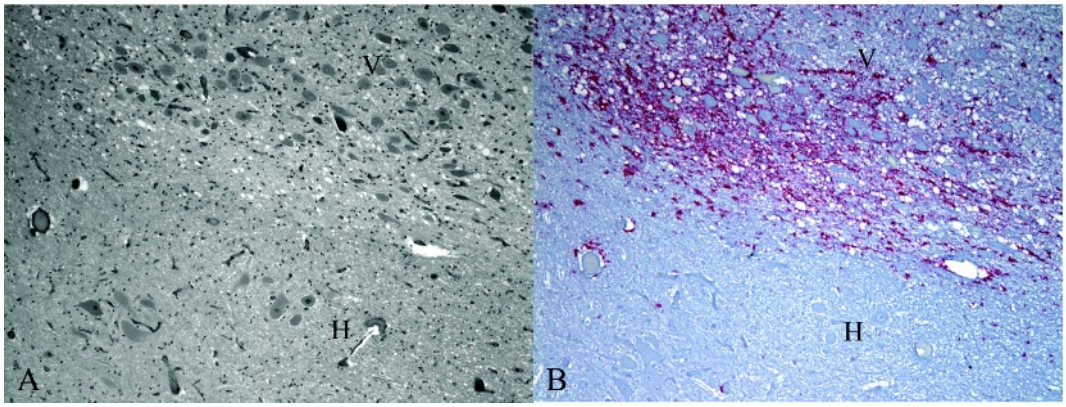

FIGURE 1. Terminal brain stem at the level of the obex. A. Spongiform change affecting the neuropil is prominent in the dorsal motor nucleus of the vagus nerve (V). Similar spongiosis is lacking in the hypoglossal nucleus. H&E stain. B. Replicate section of example A. Marked accumulation of PrP^{CWD} in the dorsal motor nucleus of the vagus nerve (V). Minimal staining is evident in the hypoglossal nucleus (H). Alkaline phosphatase immunohistochemistry with Fast Red chromagen.

neuropil (Fig. 1B). At this level of the brain stem, perivascular as well as plaque-like staining in the neuropil occurred in the median raphe, radix of the vagus nerve, spinal nucleus, and tract of the trigeminal nerve and in reticular formation. Deposits of bright red chromagen occurred around rare large neurons in reticular areas. At the level of the mesencephalon, coarse granular deposits of chromagen were scattered in the periaqueductal gray matter, reticular areas, and substantia nigra. Deposits of chromagen were also evident around a few neurons in the mesencephalic nucleus of the trigeminal nerve. In sections taken at the level of the thalamus and hypothalamus just caudal to the optic chiasma, conspicuous staining occurred in the paraventricular and supraoptic hypothalamic nuclei. Here, staining was associated with neuronal cell bodies, the neuropil, and perivascular areas. Staining for PrP^{CWD} was evident in supraependymal areas and in the subependymal fiber plexus surrounding the lateral, third, and fourth ventricles as well as the mesencephalic aqueduct. This staining was most notable lining the ventral recesses of the lateral ventricle bordering the caudate nucleus. Major brain structures lacking detectable PrP^{CWD} staining included the cerebral and piriform cortices, thalamus, hippocampus, and cerebellum.

Positive IHC staining for PrP^{CWD} was evident at all levels of the spinal cord. The pattern of staining consisted of irregular granules or plaques of bright red chromagen in the neuropil of the central gray matter, being most intense in thoracic areas. The distribution of staining was similar but varied between cervical, thoracic, and lumbar segments. In cervical cord segments, positive PrP^{CWD} staining was limited to a narrow transverse band of connecting fibers in the intermediate gray matter surrounding the central canal of anterior cervical cord. No staining was evident in the more distal cervical cord sections. Positive PrP^{CWD} staining at all levels of thoracic spinal cord also was detected in the transverse band of fibers surrounding the central canal. Here, in addition, the staining extended in an interrupted fashion to the intermediolateral gray matter. Positive staining further extended along the lateral aspects of the ventral horns. Limited staining was also present in the substantia gelatinosa. The thoracic pattern of positive PrP^{CWD} staining persisted into the lumbar

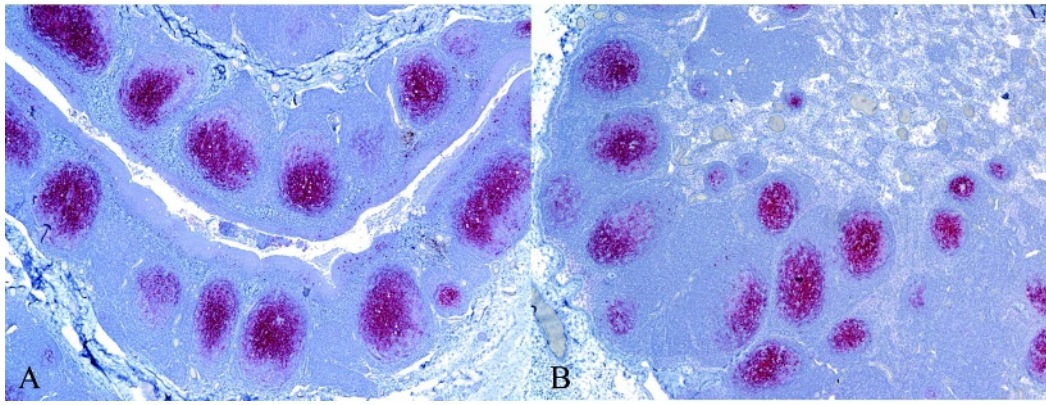

FIGURE 2. Immunohistochemistry staining for PrP^{CWD} in lymphoid tissues. Positive staining is confined mainly to lymphoid follicles. A. Tonsil. B. Medial retropharyngeal lymph node. Alkaline phosphatase immunohistochemistry with Fast Red chromagen.

cord, but involvement of the transverse fiber band surrounding the central canal was inconsistent.

In the autonomic plexuses and central peripheral ganglia, positive PrP^{CWD} IHC staining was limited mainly to granular deposits of bright red chromagen at the periphery of neuronal cells bodies and, to a lesser extent, the cytoplasm. Positive staining was detected in the myenteric plexus of the reticulum, rumen, omasum, and abomasum and in both the myenteric and submucosal plexuses in the small and large intestines. Sparse positive PrP^{CWD} IHC staining was detected in sections of nodose ganglion and dorsal root ganglia from sections of cervical and thoracic spinal cord. Staining was equivocal or undetected in stellate and trigeminal ganglia, respectively.

Strong positive PrP^{CWD} IHC staining was detected in tonsil (Fig. 2A), spleen, all lymph nodes (retropharyngeal [Fig. 2B], submandibular, superior cervical, mediastinal, mesenteric, ileocecal, pancreatic, and popliteal), and Peyer's patches of the small intestine. Staining was confined to coarse granular deposits of chromagen in lymphoid follicles. Unexpectedly, positive staining was also detected in two small peribronchial lymphoid nodules in the lung.

Positive PrP^{CWD} IHC staining was also detected in two small clusters of ventricular subendocardial Purkinje fibers of the heart and in the neurohypophysis (pituitary). No PrP^{CWD} IHC positive staining was detected in tongue, epiglottis, blood vessels, esophagus, salivary gland, liver, pancreas, urinary system, integument, bone marrow, adrenal gland, or various skeletal muscles including diaphragm.

Samples of brain, pituitary, spiral colon, spleen, multiple lymph nodes, distal ileum, and Peyer's patches also gave positive ELISA results from moose 3 (data not shown). Immunoblot analyses further verified the presence in brain and lymphoid tissues from moose 3 of PK-resistant PrP that displayed a similar electrophoretic mobility as PrP from known infected deer and elk (Fig. 3).

The DNA sequence of the PrP protein coding region showed moose 2 to be heterozygous for methionine/isoleucine at codon 209 (209MI), whereas moose 3 was homozygous for methionine (209MM). The sequence for moose 1 was not determined because of lack of suitable tissue for DNA extraction. Substitution of isoleucine for methionine at 209 occurs frequently in PrP of three subspecies of North American moose, including Shira's moose (GenBank accession No. AY225485; J. E. Jewell, unpubl. data). The PrP sequence in mule deer was 209MM, and

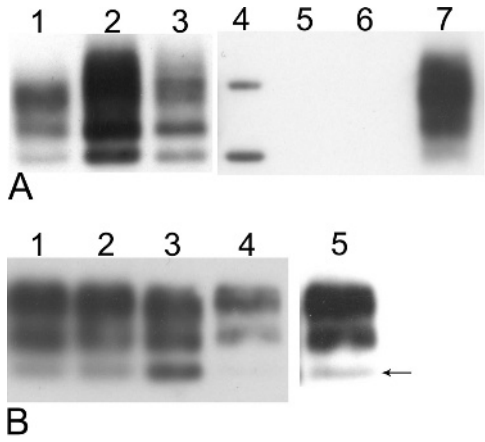

FIGURE 3. Western immunoblot of proteinase-K–resistant PrP in tissues of cervids. A. Lymphoid tissues: lane 1, CWD-infected mule deer retropharyngeal lymph node (RLN); lane 2, moose 3 ileocecal lymph node; lane 3, moose 3 tonsil; lane 4, molecular mass markers, top to bottom, 30, 20 kDa; lane 5, CWD-negative moose tonsil; lane 6, CWD-negative moose RLN; lane 7, moose 3 RLN. B. Brain tissues: lane 1, CWD-infected elk; lane 2, CWD-infected white-tailed deer; lane 3, CWD-infected mule deer; lanes 4 and 5, moose 3. Lanes 1–4, 15-min exposure; lane 5, 90-min exposure of sample in lane 4 to show unglycosylated PrP band in brain sample (arrow).

except for a mule deer polymorphism found infrequently at codon 225 (GenBank Accession No. AY228473; Jewell et al., 2005), it is the same as 209MM moose PrP at the amino acid level. Because the inoculum used to challenge these moose was a pool of mule deer brain tissue, the PrP in the pool would have been largely the very same protein sequence as PrP of moose 3, but whether this facilitated transmission of CWD to moose 3 was not known.

Moose are notoriously difficult to maintain in captivity and rarely live to advanced age (Shochat et al., 1997), which might have prevented the development of clinical CWD in moose, if such were to even occur. The relatively short life span, coupled with the difficulty in locating moose quickly after they die to allow a thorough necropsy, limited the scope of this trial. Although generalized statements concerning CWD on the basis of one moose probably should be avoided, it did appear that the distribution of accumulated PrP^{CWD} in tissues was similar to that in deer and elk (Williams and Young, 1992; Spraker et al., 1997).

The detectable accumulation of PrP^{CWD} in moose tonsil and RLN suggested that these tissues might be useful for CWD surveillance in moose as for deer. It is not known, however, if PrP^{CWD} accumulates in moose tonsil or RLN during the very earliest stages of infection as documented in deer (Sigurdson et al, 1999; Miller and Williams, 2002).

It is unknown whether moose can die from PrP^{CWD} infection and whether CWD presents a threat to free-ranging moose populations. Nonetheless, this experiment did confirm that 1) moose can become orally infected with mule deer–derived CWD, 2) PrP^{CWD} propagated and accumulated in multiple lymphoid and nervous tissues similar to deer and elk (Spraker et al., 1997), and 3) PrP^{CWD} caused spongiform changes in the central nervous system considered characteristic for CWD in cervids (Williams and Young, 1992). Thus, this is the first report of experimental CWD in moose.

Subsequent to these findings, a hunter-killed wild moose was diagnosed with CWD in Colorado in September 2005 (M. Miller, unpubl. data). Although CWD has now been established in moose, cases in moose probably will be a rare occurrence because their social habits differ from elk and deer. Nonetheless, wildlife managers should be aware of this possibility and increase their surveillance efforts for naturally occurring CWD in moose.

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