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# OPPORTUNISTIC SURVEILLANCE OF CAPTIVE AND FREE-RANGING BIGHORN SHEEP (*OVIS CANADENSIS*) IN COLORADO, USA, FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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ABSTRACT: Bighorn sheep (*Ovis canadensis*) are predicted to have a degree of susceptibility to the transmissible spongiform encephalopathies (TSE) chronic wasting disease and scrapie. We opportunistically screened 127 captive bighorn sheep and 152 free-ranging bighorn sheep in Colorado, US for the presence of TSE over a period of 35 yr. None of the animals demonstrated clinical signs, gross pathology, histopathology, or immunohistochemical staining patterns suggestive of TSE.

*Key words:* Bighorn sheep, chronic wasting disease, immunohistochemistry, *Ovis canadensis*, scrapie, transmissible spongiform encephalopathy.

## INTRODUCTION

Chronic wasting disease (CWD) was first diagnosed as a transmissible spongiform encephalopathy (TSE) of captive mule deer (*Odocoileus hemionus hemionus*) and black-tailed deer (*Odocoileus hemionus columbianus*) in Colorado and Wyoming, US in 1978 (Williams and Young 1980). The disease was later recognized in elk (*Cervus canadensis*; Williams and Young 1982; Spraker et al. 1997), white-tailed deer (*Odocoileus virginianus*; Spraker et al. 1997), and moose (*Alces alces*; Baeten et al. 2007). North American caribou (*Rangifer tarandus*) were also shown to be susceptible to CWD by oral inoculation (Mitchell et al. 2012), confirming the occurrence of, or potential for, CWD infections across all species of Cervidae native to North America. Chronic wasting disease has been detected in at least 30 states and provinces in the US and Canada (CWD Info 2020). Free-ranging Cervidae have been diagnosed with CWD in northern Europe, including reindeer (*Rangifer tarandus*), moose, and red deer (*Cervus elaphus*; Benestad et al. 2016; Pirisinu et al. 2018; Vikøren et al. 2019). There are concerns for introduction of CWD by importation as occurred in South Korea (Kim et al. 2005), where CWD was detected in red deer and sika deer (*Cervus nippon*) after importation of CWD-infected elk (Lee et al. 2013). In addition to morbidity and mortality of individuals, population-limiting effects of the disease are also being recognized in areas where CWD is prevalent (Edmunds et al. 2016; DeVivo et al. 2017).

Documented natural cases of CWD are limited to the family Cervidae. However, the potential susceptibility of other, more distantly related species has been suggested by intracerebral inoculation studies and protein conversion assays. Domestic sheep (*Ovis aries*) are susceptible to CWD by intracerebral inoculation (Hamir et al. 2006), and both domestic and bighorn sheep (*Ovis canadensis*) are potentially susceptible to CWD on the basis of protein conversion assays (Raymond et al. 2000; Morawski et al. 2013). Species phylogeny and *PRNP* (prion protein) gene polymorphisms also predict susceptibility of bighorn sheep to CWD (Cullingham et al. 2020). Bighorn sheep overlap in range with deer, elk, and moose in parts of western North America, raising concerns for the transmission of CWD from cervids to bighorn sheep (Morawski et al. 2013). Additionally, bighorn sheep may be exposed to scrapie, a naturally

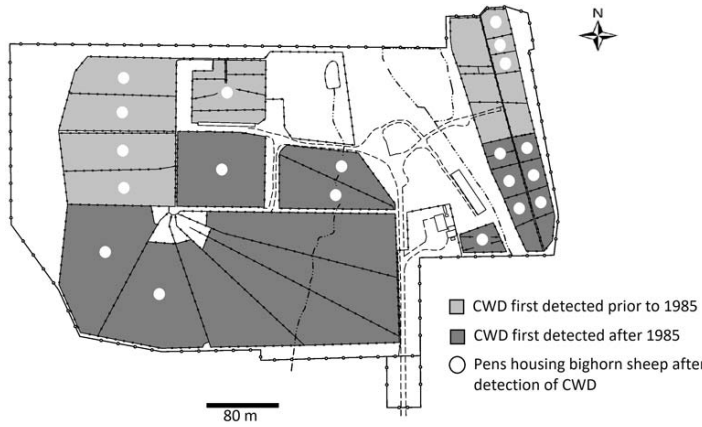


FIGURE 1. Distribution of naturally occurring and experimental cases of chronic wasting disease (CWD) in bighorn sheep (*Ovis canadensis*) at the Colorado Parks and Wildlife Foothills Wildlife Research Facility (Fort Collins, Colorado, USA). The light gray areas represent pens where cases of CWD were first detected before 1985, and the dark gray areas represent pens where CWD was first detected after 1985. Bighorn sheep were introduced to the facility in 1985 after CWD eradication efforts. White dots represent pens where bighorn sheep have been housed after detection of CWD in the pen.

occurring TSE of domestic sheep and goats (*Capra hircus*; Greenlee 2019) that has also been recognized in captive mouflon (*Ovis orientalis orientalis*) sheep (Wood et al. 1992a). For these reasons, surveillance of bighorn sheep for TSE may be warranted, particularly in areas with high current or historical risk for exposure. Here we describe opportunistic TSE surveillance in bighorn sheep from a captive facility with enzootic CWD and in free-ranging bighorn sheep over a period of 35 yr.

## MATERIALS AND METHODS

We conducted opportunistic surveillance for TSE in both captive and free-ranging bighorn sheep populations. The captive population has been housed at the Colorado Division of Parks and Wildlife (CPW) Foothills Wildlife Research Facility (FWRF; Fort Collins, Colorado, USA) since 1985. Many of the first clinically recognized cases of CWD occurred at the FWRF site in the 1960s (Williams and Young 1992), and the earliest diagnoses of CWD as a TSE included cases from this facility (Williams and Young 1980, 1982, 1992). In 1985, an effort was made to eradicate CWD from the facility through culling of all cervids, ground treatment with calcium hypochlorite, plowing of the ground, repeated treatment with calcium hypochlorite, and installation of fencing to prevent contact with free-ranging cervids (Miller et al. 1998). Captive bighorn sheep were transferred to the facility in 1985 after CWD eradication efforts, and bighorns were housed in some of the same pens that previously had held CWD-infected deer and elk (Fig. 1). Elk calves were reintroduced to the facility in 1986, and CWD recurred by 1989 (Miller et al. 1998). Mule deer fawns from nonenzootic areas were reintroduced to the facility beginning in 1990, with cases of CWD noted in these deer by 1994 (Miller and Wild 2004). White-tailed deer fawns from a nonenzootic location were introduced in 1993, with CWD observed in these deer by 1997 (Miller and Wild 2004). Since 1986, bighorn sheep, deer, and elk have shared the facility, with spatiotemporal overlap as shown in Figure 1. The facility has been heavily used for CWD transmission studies (Miller and Williams 2003; Wolfe et al. 2012; Williams et al. 2018), and natural cases of CWD continue to occur sporadically in susceptible cervid species housed at the facility. For these reasons, the FWRF appears to be a potentially high-risk environment for natural transmission of CWD to bighorn sheep.

Before about 1996, we conducted TSE surveillance in captive bighorn sheep through antemortem observations for clinical signs compatible with CWD (Williams and Young 1992) or scrapie (Detwiler 1992; Wood et al. 1992a, b) and through postmortem examination of brain tissues for histologic evidence of spongiform encephalopathy (Williams and Young 1992). After the availability of immunohistochemical diagnostics for CWD (Spraker et al. 2002), bighorns that died at the facility were screened for the presence of

TSE by immunohistochemistry (IHC) of lymphoid tissue, brainstem at the level of the obex, or both. Immunohistochemical diagnostics since 2002 consistently used the commercially available F99/97.6.1 antibody (Veterinary Medical Research and Development, Pullman, Washington, USA). This antibody has been shown to detect the resistant prion proteins of TSE, including CWD (Spraker et al. 2002) and scrapie (O'Rourke et al. 2000), as well as the proteinase-resistant prion protein detected in sheep that developed clinical scrapie after intracerebral inoculation with CWD (Hamir et al. 2006). All IHC was performed by an accredited diagnostic laboratory (Colorado State University, Fort Collins, Colorado, USA, or Wyoming State Veterinary Laboratory, Laramie, Wyoming, USA).

To augment data from captive bighorn sheep, we also screened free-ranging bighorn sheep from throughout Colorado for evidence of TSE or prion infection. For this effort, we opportunistically collected samples from carcasses submitted to the CPW Wildlife Health Program for postmortem examination.

## RESULTS

From 1985 to 2020, captive bighorn sheep ($n$=127) at the FWRF were observed for clinical signs of TSE. Animals included in this total were present at the facility for at least 1 yr and included 70 female, 55 male, and 2 male castrated bighorns, with ages ranging from 1 to 21 yr (mean 6.2 yr, median 5 yr) at the time of death or transfer from the facility. Thirty animals were transferred out of the facility before death and were clinically normal at the time of transfer. None were reported as showing clinical or postmortem evidence of prion disease after the time of transfer. Of the 97 captive bighorns resident at the FWRF for >1 yr and examined postmortem, causes of death at the facility included disease ($n$=47), terminal study animal ($n$=22), trauma ($n$=13), capture mortality ($n$=7), herd management ($n$=6), and unknown ($n$=2). Clinical signs of ill thrift, weight loss, poor hair coat, incoordination, paresis, stargazing, or other neurologic deficits were rarely noted, and ultimately such signs were attributed postmortem to respiratory disease, bluetongue, gastrointestinal disease, or trauma, with no postmortem findings suggestive of TSE diseases.

Before 2003, available records from the facility are limited, and some results are provided to the best knowledge of those on staff during that time. From 1985 to 2020, an estimated 17 bighorns held at the facility for at least 1 yr were screened for TSE postmortem only by histologic examination of brain tissue ($n$=12 confirmed by records). An estimated 29 bighorns held at the facility for at least 1 yr were screened for TSE postmortem by both histologic examination of the brain and IHC of brain or lymphoid tissues ($n$=7 confirmed by records). A total of 42 bighorns held at the facility for at least 1 yr were screened for TSE by IHC only (all 42 confirmed by records). For animals with records available, the samples evaluated by IHC for each individual included brainstem at the level of the obex and retropharyngeal lymph node ($n$=31); retropharyngeal lymph node only ($n$=9); brainstem only ($n$=5); brainstem and tonsil (n=2); and brainstem, retropharyngeal lymph node, and tonsil ($n$=2). No lesions of spongiform encephalopathy were observed by histologic examination of the brain. Rare observations of perikaryonic vacuoles unassociated with prion protein deposition were not consistent with TSE (Gould et al. 2003). None of the tissues examined demonstrated IHC staining suggestive of TSE diseases.

In addition to long-term monitoring of our captive bighorn herd, from 2003 to 2020, we tested 152 free-ranging bighorn sheep by IHC for the presence of prion diseases. Animals included 98 female and 54 male bighorns >2 yr old. Exact ages were not available for free-ranging animals. Tested samples for each individual included brainstem at the level of the obex and retropharyngeal lymph node ($n$=104), brainstem only ($n$=24), retropharyngeal lymph node only ($n$=22), or tonsil only ($n$=2). Causes of death included roadkill ($n$=59), unknown ($n$=19), disease ($n$=15), capture mortality (n=12), interaction with domestics ($n$=12), cull because of presence of disease ($n$=11), predation ($n$=8), harvest ($n$=5), fall from cliff ($n$=4), other trauma ($n$=5), poaching ($n$=1), and winter starvation ($n$=1). Free-ranging bighorns that were tested originated from 35 of the 85 bighorn sheep

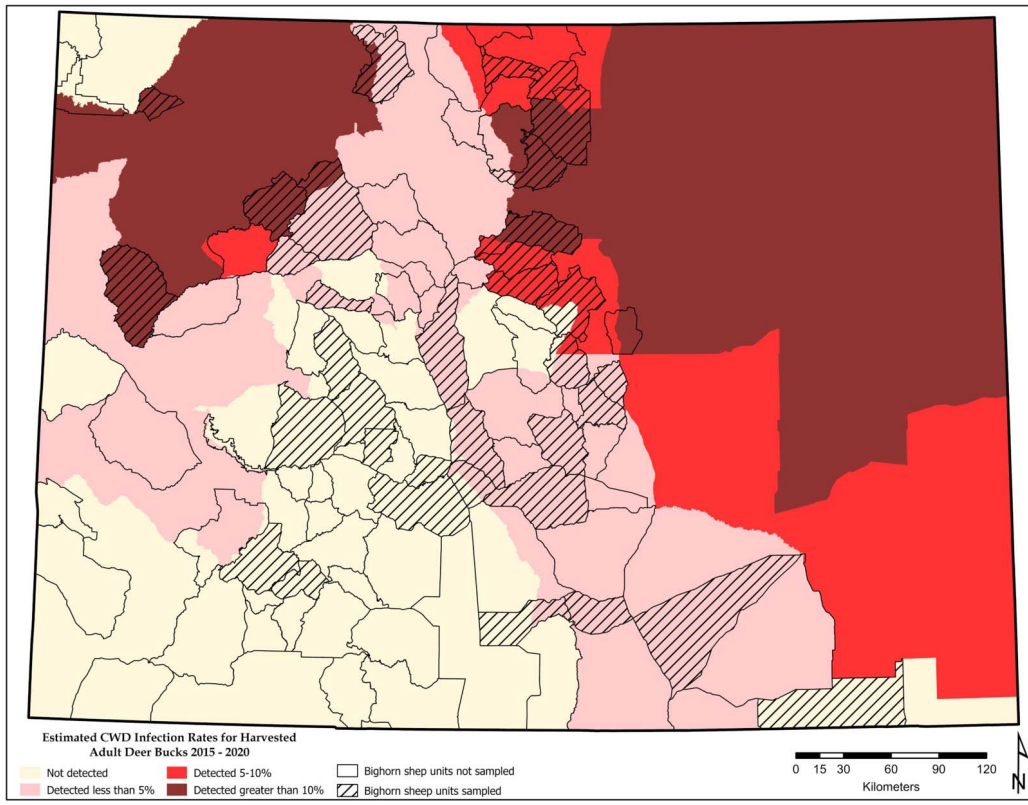


FIGURE 2. Overlap of bighorn sheep (*Ovis canadensis*) management units with naturally occurring chronic wasting disease (CWD) in Colorado, USA. Black solid outlines denote bighorn sheep management units. Shaded areas demonstrate areas where chronic wasting disease is enzootic in free-ranging cervids; darker colors represent higher CWD prevalence. Hatched areas represent bighorn sheep management units that were sampled as part of this project.

management units in the state. Some free-ranging bighorn sheep herds overlap with areas of the state where CWD is enzootic in free-ranging cervids (Fig. 2). Of the 152 tested free-ranging bighorns, 134 (88%) originated from areas with CWD detected in free-ranging deer, 97 (64%) originated from areas with >5% prevalence of CWD in free-ranging deer, and 41 (27%) originated from areas with >10% prevalence of CWD in free-ranging deer. No cases of prion disease have been detected in free-ranging bighorns.

## DISCUSSION

To date, TSE has not been detected in bighorn sheep. However, concerns for transmission of scrapie or CWD to bighorns are understandable. Bighorns are predicted to be susceptible to scrapie; they share a prion protein amino acid sequence with scrapie-susceptible sheep, and protein conversion assays do not predict a species barrier for scrapie in bighorns (Morawski et al. 2013). Evidence for the susceptibility of bighorn sheep to CWD is less extensive, although protein conversion assays do predict a low species barrier (Morawski et al. 2013), and genetic sequences from both species phylogeny and PRNP gene polymorphisms predict susceptibility to the disease (Cullingham et al. 2020). Although *PRNP* gene sequence similarities and phylogenetic relationships can help predict susceptibility to CWD, experimental transmission studies of CWD have not been pursued with bighorn sheep. The

inability to transmit CWD from a contaminated environment to fallow deer (a member of the family Cervidae with high *PRNP* gene homology to other cervid *PRNP* gene sequences; Rhyan et al. 2011; Wik et al. 2012) suggests that additional factors beyond *PRNP* gene homology and phylogeny contribute to CWD susceptibility.

Because of the highly CWD-contaminated environment of the CPW FWRF and the continuous exposure of captive animals to this environment, we determined this site to be a high-risk environment for potential transmission of CWD to bighorns. We conducted opportunistic TSE surveillance in a population of bighorn sheep housed at this facility from 1985 to 2020. There was no evidence of clinical CWD (or scrapie) in observations of 127 individual bighorns from this population over 35 yr and no evidence of prion infection in the subset of 97 bighorns examined postmortem. These data suggest that CWD is not readily transmitted to bighorns by exposure to a heavily contaminated environment.

Free-ranging populations of bighorn sheep are not expected to experience the same level of environmental exposure to CWD as the captive animals in this surveillance study, although free-ranging bighorns may occasionally have direct contact with free-ranging cervids in addition to sharing habitat. Because scrapie is regulated in the US (9 CFR parts 54 and 79), exposure of bighorns to scrapie-infected sheep or goats is expected to be minimal. However, free-ranging bighorns are more likely than captive animals to be exposed to scrapie-associated prions. There was no evidence of CWD or scrapie in 152 free-ranging bighorns evaluated over 17 yr.

Although our data suggest that CWD and scrapie are unlikely or rare in bighorns in Colorado, additional TSE surveillance may be useful in bighorn sheep and other noncervid species with predicted susceptibility to TSE diseases. Based on our findings and recommendations for detecting CWD in cervids (EFSA BIOHAZ et al. 2017), targeted surveillance for animals showing compatible clinical signs or with a high risk for exposure to TSE may be more effective than large-scale surveillance of apparently healthy populations or those with a low risk for exposure. Because this study took place over 35 yr with variable availability, validation, and consistency of diagnostic tests, we relied heavily on IHC for consistency over time. Additional surveillance may be enhanced by the use of more sensitive diagnostic tests such as enzyme-linked immunosorbent assay, protein misfolding cyclic amplification, or real-time quaking-induced conversion. Interpretation of positive results may be complicated by the current lack of known positive control tissues from bighorn sheep. Differentiation between CWD and scrapie in bighorn sheep may also be challenging on the basis of minimal variation in western blot patterns from natural cases of scrapie and experimental cases of CWD in sheep (Hamir et al. 2006) and ovinized transgenic mice (Madsen-Bouterse et al. 2016).

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