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CATTLE (*BOS TAURUS*) RESIST CHRONIC WASTING DISEASE FOLLOWING ORAL INOCULATION CHALLENGE OR TEN YEARS' NATURAL EXPOSURE IN CONTAMINATED ENVIRONMENTS

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ABSTRACT: We conducted a 10-yr study to establish whether chronic wasting disease (CWD) was readily transmissible to domestic cattle (*Bos taurus*) following oral inoculation or by cohousing cattle with captive cervids in outdoor research facilities where CWD was enzootic. Calves (n=12) were challenged orally on one occasion using brain homogenate derived from CWD-infected mule deer (*Odocoileus hemionus*). Five uninoculated cattle served as unchallenged controls. Two other groups of cattle (n=10–11/group) were housed outdoors for 10 yr in captive cervid research facilities. The environmentally challenged cattle were exposed to CWD-associated prions through common paddocks, feed, and water and via direct daily contact with known and potentially infected mule deer or wapiti (*Cervus canadensis*) throughout the decade-long study period. None of the exposed cattle developed neurologic disease during the study. We euthanized cattle surviving to 10 yr postchallenge and examined all for lesions or disease-associated prion protein (PrP^d) by histopathology, immunohistochemistry, and western immunoblot analysis of central nervous system and lymphoid tissue. None had evidence of PrP^d accumulation. We conclude that the risks of CWD transmission to cattle following oral inoculation or after prolonged exposure to contaminated environments are low.

Key words: *Bos taurus*, cattle, *Cervus canadensis*, chronic wasting disease, mule deer, *Odocoileus hemionus*, prion, wapiti.

INTRODUCTION

Prion diseases of domestic and wild ruminants have generated global concern because of their implications for animal production, international trade, and human health. The study described here was prompted by questions about whether chronic wasting disease (CWD; Williams and Young 1980) of North American cervids could be transmitted to domestic cattle (*Bos taurus*) via natural exposure routes. Worry over the possibility that CWD-infected wildlife could transmit prions to cattle was kindled by epidemiologic evidence that the bovine spongiform encephalopathy (BSE) outbreak in the UK in the 1980s and 1990s might have originated from cattle becoming infected with the sheep scrapie agent (Wilesmith et al. 1988, 1991). The BSE epizootic was an extended, common-source epidemic driven by feeding contaminated meat and bone meal-derived feed supplements, the true origin of which remains unclear (Prince et al. 2003; Konold et al. 2013). However, the emergence of a variant form of Creuztfeldt-Jacob disease in humans linked to the BSE agent (Bruce et al. 1997) challenged the well-established tenet that prion diseases in other animals did not transmit to humans. This heightened concern about prospects for a BSE-like outbreak should CWD prions be naturally transmissible to cattle.

The circumstances giving rise to the BSE outbreak in the UK are largely irrelevant to assessing the risk of cattle exposure to CWD

in North America. Cervid carcasses have not been rendered or used in meat and bone meal intended for ruminant feed since the late 1990s. However, cattle may be exposed to the CWD agent by grazing on shared pastures or ranges and by consuming hay or other crops harvested from fields where CWD-infected cervids forage and could be exposed to infected carcass remains. Such exposure likely would be a byproduct of the horizontal transmission through direct or environmental exposure—including contaminated feed and water sources—that appears to be the primary driver of CWD epizootics among susceptible cervid species (Miller et al. 2000; Miller and Williams 2003; Williams 2005).

Here, we report findings from three, interrelated 10-yr studies that examined susceptibility of cattle to cervid prions after oral inoculation with infectious mule deer (*Odocoileus hemionus*) brain tissue or after cohabitation with infected mule deer or wapiti (*Cervus canadensis*). Preliminary negative clinical findings of the oral challenge study were reported briefly in a previous review of CWD (Williams 2005), and CWD transmission to sentinel mule deer was described by Miller and Williams (2003).

MATERIALS AND METHODS

We used two approaches to assess natural susceptibility of cattle to CWD under experimental conditions. The first involved exposing healthy, weanling, beef breed calves to a large, single, oral dose of the CWD agent and maintaining them in isolation for 10 yr. The second involved confining calves in paddocks with CWD-infected mule deer or wapiti for 10 yr and monitoring them for clinical prion disease. In addition to observing exposed cattle for clinical disease for a decade, we collected tissues postmortem and analyzed them for presence of disease-associated prion protein (PrP^d). We chose the 10-yr assessment period largely for logistical reasons while anticipating a protracted incubation period for cross-species transmission (e.g., 6 yr for cattle intracerebrally inoculated with CWD; Hamir et al. 2005).

Cattle

In June 1997, the Colorado Division of Wildlife (CDOW) purchased 59 mixed beef breed (mainly Hereford × Angus) calves about 4 mo old of both sexes from a Wyoming, US ranch outside the area where CWD was known to occur at that time (Miller et al. 2000). We transported them to the Wyoming State Veterinary Laboratory (WSVL; Laramie, Wyoming, USA). Calves received standard vaccinations (for bovine viral diarrhea virus, bovine herpesvirus 1, bovine parainfluenza 3 virus, bovine respiratory syncytial virus, and for *Clostridium* spp.) and antihelmintics. Male calves were castrated. After brief acclimation, calves were randomly divided among four animal research facilities operated by the University of Wyoming (WSVL), the CDOW (Foothills Wildlife Research Facility, Fort Collins, Colorado, USA), the Wyoming Game and Fish Department (WGFD; Sybille Research Facility, Wheatland, Wyoming, USA), and the US Department of Agriculture, Agricultural Research Services (National Animal Disease Center [NADC], Ames, Iowa, USA).

Eighteen calves sent to the NADC were used in an intracerebral (IC) challenge study described elsewhere (Hamir et al. 2001, 2005). We randomly assigned the 41 remaining calves to one of four experimental groups described below. All procedures were approved by respective institutional animal care and use committees (CDOW file 1997-2; WSVL file unnumbered).

Oral inoculation

Twelve calves were held indoors at WSVL. On 26 August 1997, each calf received a single oral dose of 45 g of pooled, homogenized tissue macerate derived from the brains of 28 CWD-infected mule deer collected from CDOW and WGFD facilities. The specific infectivity of the inoculum has not been measured by bioassay, but Raymond et al. (2000) estimated the PrP^d concentration to be about 3 μg/g tissue pool. This pool readily infected mule deer challenged orally, with a complete attack rate produced using as little as 1 g delivered in a single dose (Fox et al. 2006; Miller et al. 2012). This tissue pool also was the source of material used for cell-free conversion assays of potential cattle susceptibility to CWD (Raymond et al. 2000) and for IC inoculation of cattle at the NADC (Hamir et al. 2001, 2005). Five additional calves were maintained separately outdoors at the WSVL and served as unexposed (negative) controls.

Orally inoculated cattle were housed two per room in an isolation building at the WSVL. They were fed alfalfa hay and pelleted growth and maintenance rations containing no ruminant protein. Clean water and mineral licks were available ad libitum. Personnel wore protective clothing while in the facility. Animals were observed and pens cleaned daily. All wastes were

incinerated on site. We made routine veterinary observations regularly during the course of the 10-yr isolation, with emphasis on detecting signs consistent with prion disease in cattle: altered temperament or behavioral changes, abnormal mental status, hyperesthesia, changes in posture or gait such as ataxia, incoordination or proprioceptive deficits, nystagmus, head tilt, head tremor, paresis, and weight loss (Wells et al. 1987; Wilesmith et al. 1988; Hamir et al. 2001). Controls received the same diet and also were observed regularly.

Exposure via cohabitation

Twelve calves each were transported to WGFD or CDOW facilities on 24 June or 1 July 1997, respectively, and kept in outdoor paddocks. Depending on study site, the source of prion exposure was mule deer (CDOW facility) or wapiti (WGFD facility). To maximize the likelihood of exposure to CWD-associated prions, cattle shared common paddocks, feed, and water with infected cervids and had opportunity for direct contact with infected deer or wapiti throughout the study period. All paddocks used in both facilities had previously or concurrently held cervids naturally or experimentally infected with CWD as part of other studies. Cattle were regularly rotated through the study paddocks. In the CDOW facility these were paddocks A, B1, and B2 as described previously (see fig. 2 in Miller and Wild 2004). Cattle and syntopic cervids received native forage in addition to grass hay, grain-based pelleted feed, and mineral-salt supplement. Cattle at the CDOW facility were excluded from some feeding and bedding areas to protect cohoused deer from underfeeding, malnutrition, and harassment, but the exclosures were opened to cattle periodically. Each day the cattle consumed all feed left over by deer.

Deer or wapiti were added to the respective exposure systems by both natural birth and supplemental stocking throughout the 10-yr study period. The study design was to maintain about a 1:1 ratio of known and potentially infected cervids to cattle. We recorded dates in and out of study paddocks to aid in estimating the magnitude of potential exposure. Cattle and cohoused cervids were observed daily for evidence of clinical disease.

To provide evidence for ongoing prion transmission in the paddocks where cattle were confined in the respective facilities, we captured sentinel mule deer (n=11; Miller and Williams 2003) or wapiti (n=18) from populations where CWD did not occur and held those animals in the same paddocks as the cattle. The sentinel animals served as exposure controls and later as sources of infectivity.

Postmortem and laboratory assessment

Deer and wapiti dying or euthanized during the course of the study were necropsied and screened microscopically for evidence of prion infection using diagnostic procedures, including immunohistochemistry (IHC), using methods described by Miller and Williams (2002).

Thirty-four cattle survived to the end of the 10-yr study period. They were euthanized in staggered groups for logistical reasons (10 July–8 August 2007 for CDOW group; 14 August–13 September 2007 for WGFD group; 24 September 2007–27 August 2008 for WSVL group). All cattle other than those housed at the WSVL were transported to WSVL. There, each was sedated with xylazine (about 500 mg) and killed via intravenous overdose of sodium pentobarbital. Carcasses were necropsied immediately after death. We fixed tissues in 10% neutral buffered formalin and froze duplicate samples at –20 C or –80 C.

We fixed central nervous system (CNS) tissues for 14 d and nonneurologic tissues for 7 d prior to trimming tissue blocks. Trimmed tissues were immersed in 98% formic acid for 1 h and rinsed in tap water for 24 h. To minimize vacuolation artifact, CNS tissues were processed into wax using a 19-h protocol as follows: 9 h in ascending concentrations of alcohol, 4.5 h in Pro-Par (Anatech Ltd., Battle Creek, Michigan, USA) clearing solution, and 5.5 h in paraffin wax (Wells and Wells 1989). Tissues were embedded in paraffin wax, sectioned at 5–6 μm, and stained with H&E using standard histologic techniques.

Immunohistochemical staining for PrP^d was performed following published procedures (Manning et al. 2008; see Supplementary Materials). This protocol detected PrP^d in known-positive brain tissue blocks from cattle challenged IC with CWD brain homogenates (Hamir et al. 2005).

We performed IHC for PrP^d on tissues of necropsied cattle using standardized levels (areas or sites) of brain, spinal cord, lymphoid and endocrine systems, and visceral organs. These included 13 levels of brain at the following locations: medulla oblongata at rostral aspect of central canal, at obex and at cerebellar peduncles; cerebellum at cerebellar roof nuclei and through vermis; posterior and rostral colliculi; hippocampus; thalamus at mammillary body and optic tract; basal ganglia and caudate nuclei; and occipital, temporoparietal, and frontal cortex. Spinal cord was examined by IHC at spinal nerve segments C3, C4, T9, T10, L2, and L3. Systemic tissues examined by IHC included submandibular, medial retropharyngeal, and mesenteric lymph nodes and palatine tonsil. Additional samples from the orally challenged group and from uninoculated controls examined by IHC included lymph nodes,

spleen, ileum with gut-associated lymphoid tissue (Peyer's patches), and three skeletal muscles (musculus longissimus lumborum, semitendinosis, triceps brachii). All H&E and IHC sections were evaluated by one pathologist (D.O'T.) using criteria for diagnosing transmissible spongiform encephalopathies, particularly lesions in BSE-infected cattle (e.g., Wells et al. 1991; Wells and Wilesmith 1995; Konold et al. 2012), scrapie-infected cattle (e.g., Cutlip et al. 1994; Clark et al. 1995; Hamir et al. 2011), CWD-infected cattle (e.g., Hamir et al. 2005, 2006, 2007; Greenlee 2012), and CWD-infected deer, wapiti, and moose (e.g., Williams and Young 1993; Williams 2005; Kreeger et al. 2006).

To further assay brain tissues from the 34 longest surviving cattle, samples were generated for gel electrophoresis and subsequent western blot analysis. Samples from gels were transferred to Immobilon-P PVDF membranes (Millipore, Billerica, Massachusetts, USA) and western immunoblot development was carried out as previously described (Jewell et al. 2006; see Supplementary Materials).

To assess the genetic makeup of study cattle, we extracted genomic DNA from frozen spleen or lymph node tissue using the DNeasy kit (Qiagen, Valencia, California, USA) and manufacturer's protocol. Prion protein coding sequences were amplified by PCR using conditions and PCR primers as described in Jewell et al. (2005), and DNA was sequenced from total PCR product (Amplicon Express, Pullman, Washington, USA) using modified primer 12(bov), 5′ TGG TGG TGA CTG TGT GTT CCT TGA 3′, and primer 3FL1. Sequence results were analyzed using Chromas (Technelysium Pty. Ltd., South Brisbane, Queensland, Australia) and DNASTAR (DNASTAR Inc., Madison, Wisconsin, USA) software.

RESULTS

Thirty-four of 41 cattle survived to the end of the 10-yr study period. Between 1997 and 2007, seven cattle died of intercurrent disease unrelated to prion exposure. Causes of death included lightning strike, traumatic reticuloperitonitis, and foreign body ingestion (plastic bag). Of these seven animals, two were oral challenge animals housed indoors, three (one at the CDOW and two at the WGFD facility) were exposed via cohabitation, and two were untreated controls. None had clinical signs consistent with prion disease or had gross, microscopic, or IHC evidence of prion infection. Otherwise, cattle held at all three facilities remained clinically healthy throughout the study period.

In contrast, nine of 11 sentinel mule deer became infected and succumbed to CWD between November 1999 and March 2005 at the CDOW facility (Fig. 1A). In addition to the sentinels, 78 other infected mule deer resided in the cattle paddocks for varying lengths of time during the study. On average, we maintained a mule deer:cattle ratio of ~1.4:1 throughout. Assuming an average disease course of 676 d from infection to death (model-averaged estimate from Miller et al. 2006) and prion shedding during the last 405 d (60% of the disease course; proportion extrapolated from data in Tamgüney et al. [2009] and Miller et al. [2012]), we conservatively estimated cattle at the CDOW facility had opportunity for >28,000 "infectious deer days" of prion exposure in addition to residual environmental contamination that accumulated before and during the study (Fig. 1A).

Similarly, all 18 of the sentinel wapiti at the WGFD facility became infected with CWD (Fig. 1B). Four developed clinical CWD between December 1999 and June 2000; the remainder were in early clinical (n=7) or preclinical (n=7) disease when euthanized in late September 2000. In addition to sentinels, 65 other infected wapiti resided in the cattle paddocks for varying lengths of time during the study. The design wapiti:cattle ratio was not maintained consistently. However, using the foregoing assumptions on disease course and shedding, we conservatively estimated that cattle at the WGFD facility had opportunity for >26,000 "infectious wapiti days" of prion exposure in addition to residual environmental contamination (Fig. 1B).

Pre-euthanasia clinical assessments of the 34 cattle surviving to the study's end revealed no abnormalities in behavior or general body condition. No unusual apprehension, gait ataxia, tremor, muscle fasciculation, hyperreaction to sound, or other typical neurologic signs associated with prion disease were observed in any of the cattle. Mild exophthalmia was present in two animals. Body condition of cattle confined indoors was

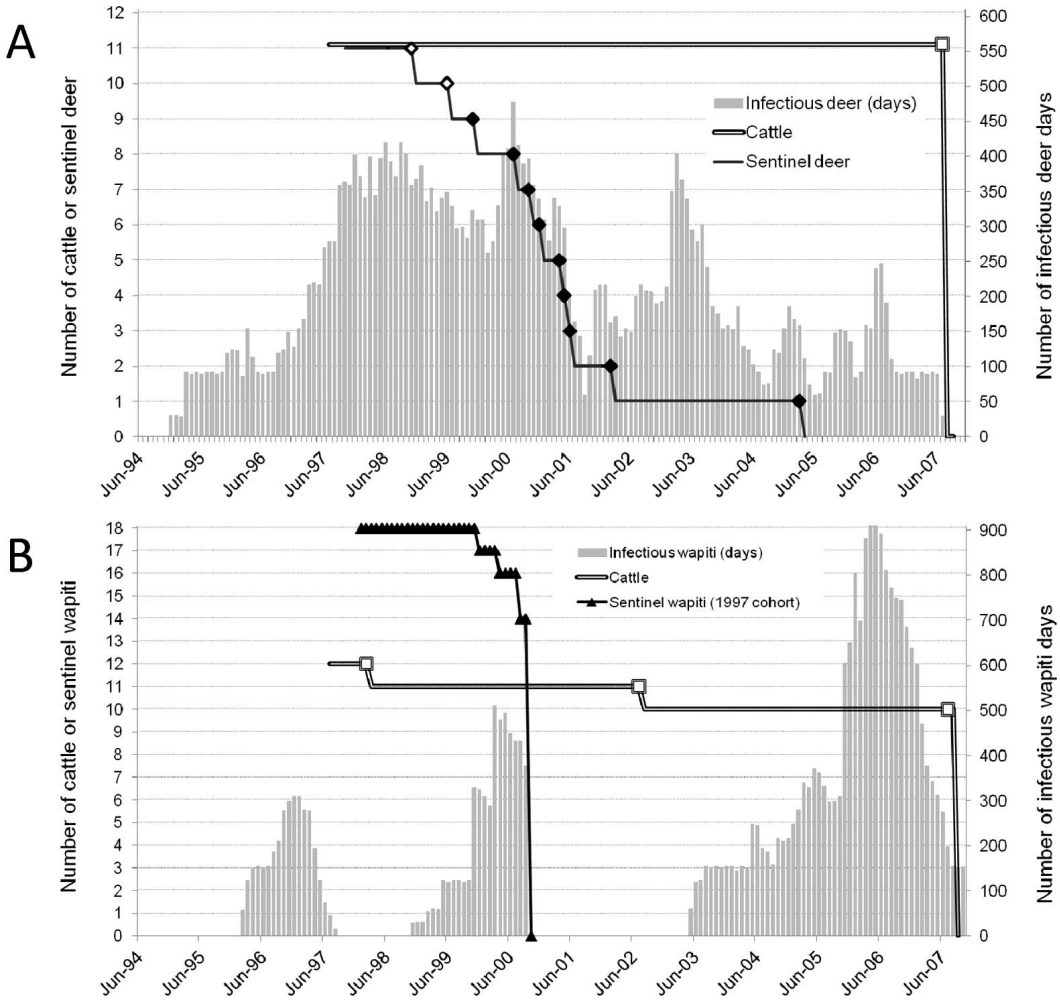

FIGURE 1. Temporal relationships between the addition and removal of domestic cattle (*Bos taurus*; *n*=24) and "sentinel" (A) mule deer (*Odocoileus hemionus*; *n*=11) or (B) wapiti (*Cervus canadensis*; *n*=18) in studies of cattle susceptibility to chronic wasting disease. No cattle showed clinical or laboratory evidence of prion infection when they died or were removed 1–123 mo after the start of the study. Infections were confirmed in nine sentinel mule deer (solid diamonds); two appeared uninfected at postmortem (open diamonds). Infections also were confirmed in all 18 sentinel wapiti (solid triangles). For infection source mule deer or wapiti, the gray bars represent the number of known or potentially infected mule deer or wapiti days (number of mule deer or wapiti × number of days) of exposure each month where at least one infection source (mule deer or wapiti) was present. Panels (A) and (B) differ in *y*-axis scaling.

adequate, despite mild disuse atrophy of muscles attributable to long-term physical inactivity.

At necropsy, carcasses of the 10 orally inoculated cattle appeared in adequate nutritional condition with perirenal, epicardial, and mesenteric fat; carcasses weighed 454–590 kg. Muscles of the pelvic girdle were moderately bilaterally atrophic, which was consistent with disuse atrophy. Gross lesions in this group that were interpreted as incidental were unilateral purulent sinusitis (*n*=1), intra-abdominal fibrous adhesions (*n*=1), and a 60×40×40-mm tan mass in the uterine wall (*n*=1). Carcasses of the 21 cattle exposed through cohabitation were in good body condition, weighing 550–

850 kg. No gross lesions were present. Carcasses of three negative control cattle were in good to obese condition (720–860 kg), with necrosis and fibrosis of mesenteric adipose tissue in one. No other gross lesions were present.

Histopathologic examination of H&E-stained brain and spinal cord sections did not reveal spongiform change (neuronal vacuolation or neuropil spongiosis) typical of the CNS lesions seen in prion diseases. Incidental microscopic findings comprised vacuolation of habenular nuclei, mild vacuolation of white matter, and intracytoplasmic vacuolation of neurons in red nucleus. Histopathology of gross lesions found at necropsy revealed a uterine leiomyoma, extensive chronic fat necrosis and fibrosis in mesenteric fat, and unilateral purulent sinusitis.

Sections from various anatomic sites in brain, spinal cord, lymphoid and endocrine systems, and viscera examined for PrP^d by IHC were negative in all experimentally exposed cattle (Fig. 2) as well as in all controls.

Using tissue taken from the ventral thalamus of each animal's brain, western immunoblots probed with anti-PrP antibody detected cellular PrP only (Fig. 3). This was present as three bands of immunoreactive protein migrating at molecular masses of about 28, 30, and 37 kDa. Comparable brain samples pretreated with proteinase K displayed no immunoreactive bands, indicating no protease-resistant prion protein. Positive controls were brain tissue homogenates from CWD-infected mule deer, which displayed the characteristic pattern of three, lower molecular weight bands persisting after proteinase K digestion (Fig. 3).

Genotyping was carried out to obtain data potentially useful in the event that animals had become infected. In the absence of that outcome, results are briefly reported here as a matter of record: The DNA sequences encoding PrP determined for all animals were in agreement with previously reported *Bos taurus PRNP* sequences available in GenBank. Three cattle had one five-repeat and one six-repeat allele, but in all others both chromosomal copies coded for the six-repeat *PRNP* allele, as is usual for domestic cattle.

DISCUSSION

Despite ample opportunity for exposure to infectivity with a decade or more for infections to develop, we observed no evidence of prion disease in any of the 31 exposed subject cattle surviving to the study's end. Lesions typical of transmissible spongiform encephalopathy were absent, including those induced by IC inoculation of the CWD agent. The observed resistance of our study cattle to CWD after exposure by natural routes and the limited susceptibility to IC challenge reported previously are consistent with a substantial species barrier to cattle propagating cervid-derived prions (Raymond et al. 2000; Hamir et al. 2005; Tamgüney et al. 2006). Our findings lend empirical support to the assessment offered by Gould et al. (2003), who examined 262 brains of culled, older age cattle syntopic with free-ranging deer (*Odocoileus* spp.) and wapiti in north-central Colorado where CWD was enzootic for decades (Miller et al. 2000). That group reported no PrP^d deposition by standard IHC methods and concluded that large-scale spread of CWD from deer to cattle under natural range conditions was unlikely.

Although we studied "natural" exposure routes, the magnitude of potential exposure was extreme by design. Cattle challenged orally with infectious tissue homogenates in quantities $\geq$45$\times$ greater than those needed to infect deer orally (Miller et al. 2012) and 450$\times$ greater than that used in IC cattle inoculations (Hamir et al. 2001), and maintained for 10–11 yr, showed no diagnostic markers for prion disease including behavioral changes, spongiform change in central nervous system tissue, or protease-resistant immunoreactive PrP^d deposition. Likewise, none of the cattle penned on prion-contaminated ground in contact with either 89 infected mule deer or 83 infected wapiti over the same 10-yr period became infected based on the same diagnostic criteria. In contrast, five of 13 other cattle

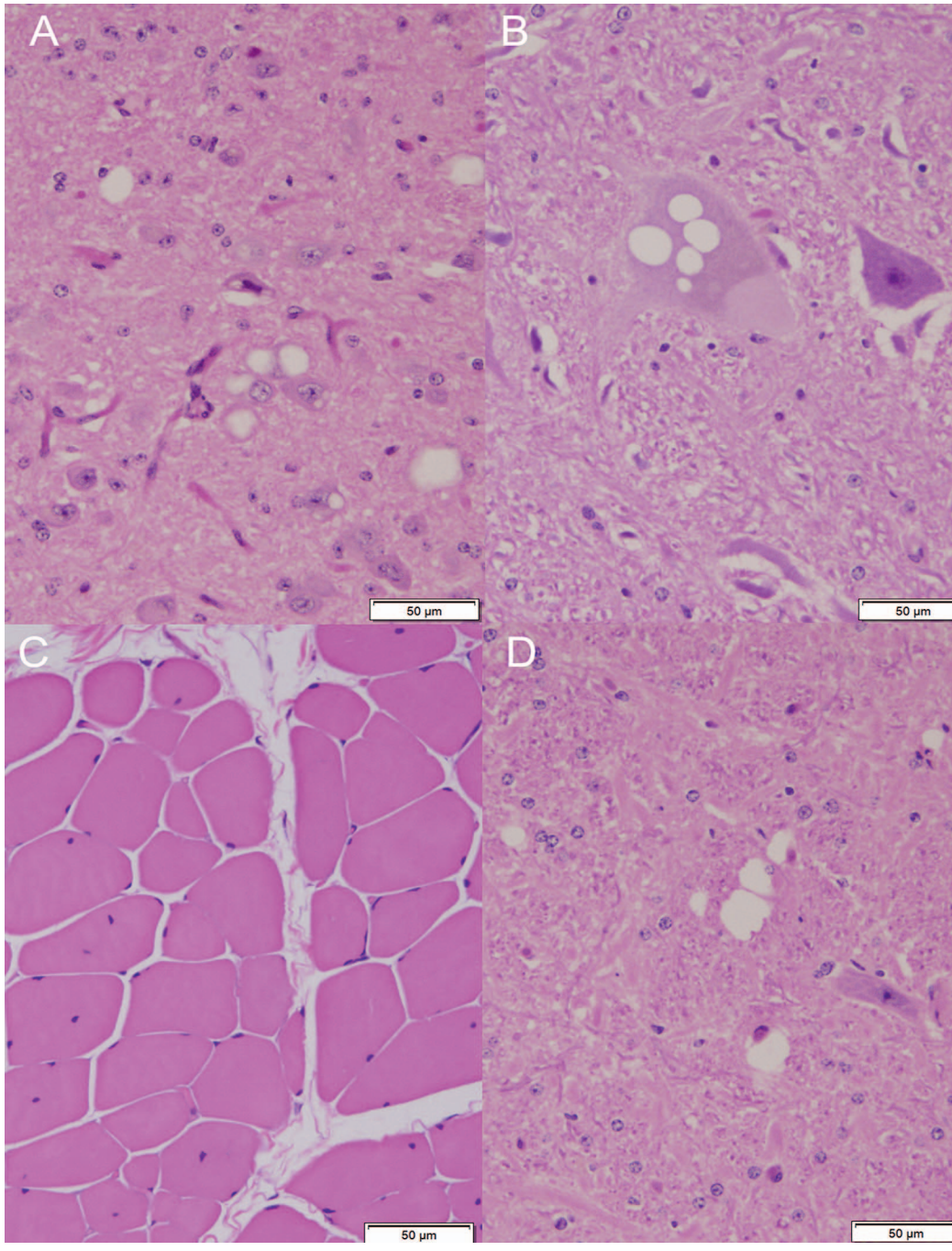

FIGURE 2. Representative incidental lesions in cattle (*Bos taurus*) following exposure to CWD either orally or by environmental challenge: (A) Intraneuronal vacuolation of habenular nucleus. H&E. 200×. (B) Intraneuronal vacuolation in red nucleus in midbrain. H&E. 200×. (C) Muscle atrophy in pelvic girdle consistent with disuse atrophy in confinement. H&E. 200×. (D) White matter vacuolation in medulla oblongata. H&E. 200×.

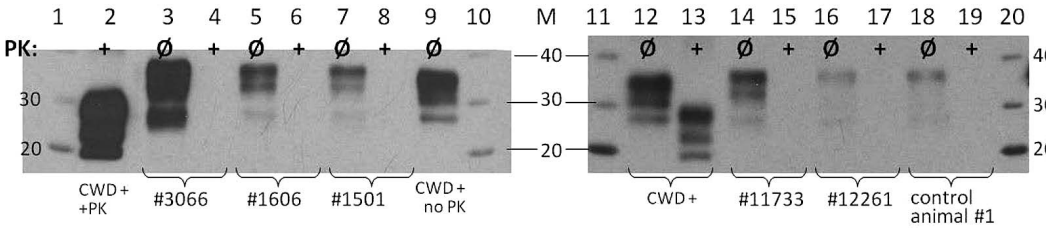

FIGURE 3. Western immunoblots probed with antiprion protein (PrP) antibody detected only cellular PrP in chronic wasting disease-exposed cattle (*Bos taurus*). Representative blots from orally inoculated cattle shown (#3066, #1606, #1501, #11733, #12261). Cellular PrP presented as three bands of immunoreactive protein migrating at molecular masses of about 28, 30, and 37 kDa when untreated with proteinase K (PK; lanes labeled with "Ø"). Comparable brain samples pretreated with PK (lanes labeled with "+") displayed no immunoreactive bands, indicating no protease-resistant prion protein. Blots (not shown) from cattle confined with infected mule deer (*Odocoileus hemionus*) or wapiti (*Cervus canadensis*) had the same outcomes. Positive controls were brain tissue homogenates from CWD-infected mule deer ("CWD+"), which displayed the characteristic pattern of three lower-molecular weight bands persisting after PK digestion.

from this cohort became infected after IC challenge using the same source inoculum (Hamir et al. 2001, 2005).

Cattle can propagate and accumulate PrP^d in brain following IC inoculation of CWD-infective material, with variable clinical expression of neurologic disease (Hamir et al. 2001, 2005, 2006, 2007, 2011; Greenlee et al. 2012). Those studies confirmed that domestic cattle are susceptible to CWD-associated prions in an absolute sense, as predicted from in vitro assays (Raymond et al. 2000). Intracerebral inoculation represents an artificial route and an overwhelming challenge that is not analogous to natural transmission. Other factors relating to interspecies transmission are likely to be circumvented by direct IC inoculation. Based on analogies with Kuru, with the suspected origins and transmission of BSE in cattle and with the suspected route of BSE transmission to wild ungulates and cats, oral inoculation should more accurately reflect a natural exposure route and host range for CWD. Intra- or interspecies CWD transmission occurred in deer, wapiti, moose, and reindeer (*Rangifer tarandus tarandus*) following oral inoculation using infective material (e.g., Williams 2005; Kreeger et al. 2006; Mitchell et al. 2012), demonstrating the value of this approach in predicting natural susceptibility of a host species (e.g., moose, reindeer; Baeten et al. 2007; Benestad et al. 2016). The logical collective inference from our and other studies is that the CNS of cattle can propagate either scrapie or CWD prions (the former more readily than the latter), but that under natural conditions—including high-dose oral exposure—transmissible spongiform encephalopathy is unlikely to develop even after an incubation period of >10 yrs.

No histologic lesions consistent with spongiform encephalopathy were induced in any of the cattle. The IHC changes of CWD following IC inoculation of cattle consist of PrP^d formation on both neurons and glial cells in a multifocal pattern in brain (Hamir et al. 2006). The range of changes found in brain and spinal cord, such as vacuolation of habenular nuclei, intraneuronal vacuolation of red nuclei, and mild white matter vacuolation were found in all groups, including unexposed cattle, and are recognized incidental changes in the brains of older cattle. This is consistent with the absence of detectable PrP^d in any of the animals.

The methods used to detect evidence of transmission (clinical signs, histologic lesions in conventional H&E-stained sections of spongiform encephalopathy, PrP^d as detected by IHC and western blot) may have missed subclinical transmission detectable by more sensitive methods such as bioassay in

susceptible laboratory animals or by protein misfolding amplification assays. These were not used, largely because our primary goal was to establish whether clinical disease and overt lesions or PrP^{d} accumulation were detectable using the standard methods available to diagnostic personnel confronted with a suspicion of a transmissible spongiform encephalopathy-like disorder in cattle in CWD-endemic areas. All of the diagnostic approaches we used have effectively detected clinical CWD in experimentally infected cattle (Hamir et al. 2011).

To date medical surveillance, epidemiologic data, and published laboratory studies have revealed no evidence of CWD transmission to humans although some potential for zoonotic risk is recognized (EFSA BIOHAZ Panel et al. 2017). Of secondary concern, passage of the CWD agent through cattle or other livestock hosts could lead to transformation of cervid-adapted prions to a new prion strain with greater potential infectivity for humans. Sheep scrapie, known for 300 yr or more, also has thus far not been linked to any human prion disease cases (EFSA BIOHAZ Panel 2015). Nevertheless, the possibility remains that a scrapie strain adapted to cattle after its inadvertent introduction became the BSE prion strain which—unlike the scrapie agent itself—eventually proved infectious to humans. Whether CWD infection of cattle would present a significant public health issue is unknown, but the potential risk would need to be evaluated. Considered in total, the improbability of cattle becoming naturally infected with CWD suggested by our and other studies should diminish concerns over these secondary transmission risks.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2017-12-299.

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