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Survey of cattle in northeast Colorado for evidence of chronic wasting disease: geographical and high-risk targeted sample

Daniel H. Gould, James L. Voss, Michael W. Miller, Annette M. Bachand, Bruce A. Cummings, Anthony A. Frank

Abstract. A geographically targeted survey of potentially high-risk, adult cattle in chronic wasting disease (CWD)–endemic areas in Colorado was initiated to assess the possibility of the spread of CWD from deer to cattle under natural conditions. Surveyed cattle were sympatric with free-roaming deer in geographically defined areas where CWD occurs and where CWD prevalence has been estimated. To qualify for inclusion in the survey, cattle had to be at least 4 years old and had to have spent a minimum of 4 years in surveyed areas. Brains from culled cattle were examined microscopically and immunohistochemically for tissue alterations indicative of a transmissible spongiform encephalopathy (TSE). Two hundred sixty-two brains were suitable for evaluation and were found to lack changes indicative of a TSE infection. Prion deposition was not demonstrable using a method involving formic acid and proteinase-K treatment before application of monoclonal antibody to bovine prion protein (F99/97.6.1). Some incidental neuropathologic changes unrelated to those of TSEs were detected. Findings from this study suggest that large-scale spread of CWD from deer to cattle under natural range conditions in CWD-endemic areas of northeast Colorado is unlikely.

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) first characterized in free-ranging and captive deer and elk in northeastern Colorado and southeast Wyoming.[13,17–19] This fatal, neurodegenerative, prion disease has been reported in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*). Since its original description, CWD has been diagnosed in other states or provinces in North America,[16] and surveillance for CWD-affected cervids is occurring in an increasing number of states. Epidemiologic evidence indicates that horizontal transmission of CWD occurs in susceptible cervids.[8] The estimated prevalence of CWD in free-ranging cervids ranges from <1% to 15%.[9]

Concern over interspecies transmission of TSEs has been heightened by the evidence linking variant Creutzfeldt–Jakob disease in humans to bovine spongiform encephalopathy (BSE).[1,3,6,12] These concerns also apply to the potential for transmission of CWD to other species, especially humans engaged in hunting, preparing, and consuming game meat. There is also concern over the potential infection of cattle. Investigation of the potential for transmission of CWD to cattle is daunting because the pathogenesis is unclear, incubation periods are prolonged, and antemortem diagnostic tests are unavailable. Confirmation of disease transmission can be accomplished only by using postmortem diagnostic techniques.

From the Departments of Pathology (Gould, Cummings, Frank), Clinical Sciences (Voss), and Environmental Health (Bachand), College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Ft. Collins, CO 80523, and the Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Ft. Collins, CO 80526 (Miller).

The current study was initiated to seek evidence for transmission of CWD to cattle by studying adult cattle residing in CWD-endemic areas of northeastern Colorado. The assumption was that this cattle population would be in contact with cervids infected with the CWD agent as well as an environment contaminated with the agent. The study required an unusual level of cooperation from a large number of livestock producers who made cattle from known geographical locations available for postmortem examination. It was surmised that if exposure of cattle to CWD actually resulted in disease transmission comparable to the levels that occur in deer, it would be possible to identify neuropathologic changes indicative of TSEs in this study population.

Cattle from 22 herds in northeastern Colorado were studied during the fall and winter seasons of 1997 and 1998. These 22 herds included 4,124 cattle, of which 262 (6.3%) were included in this study. The cattle submitted for study were often culled because of age-related problems that compromised their ability to thrive and reproduce under the extensive grazing conditions that characterize cow–calf ranching operations in the western United States. Typical old age–related problems included failure to conceive or maintain pregnancy, dental disease resulting in malnutrition, and musculoskeletal injuries. To be included in the study, cattle had to be at least 4 years old and should have lived with their herd for at least 4 years.

At culling, the following information was collected: date of culling, owner, location of herd in summer and winter, age, time in the herd, breed, sex, individual ear-tag number, and special markings or problems if relevant. Culled cattle were killed at cooperating abattoirs, and their heads were submitted to the Colorado State University Veterinary Diagnostic Laboratories, Ft. Collins, Colorado, within 6 hours of slaughter.

At the laboratory, the age recorded on the submission record was verified by evaluating replacement and wear of lower incisor teeth of each cow (Dr. Franklyn H. Garry and colleagues, Department of Clinical Sciences, Colorado State University). The calvaria were detached, the meninges were incised, and the brains were removed by severing the cranial nerves. The brain was preserved in 10% neutral buffered formalin. Sites for histopathologic analysis were trimmed, processed, and embedded in paraffin within 14 days. Select neuroanatomic areas were sectioned at 4–5 μm, stained with hematoxylin and eosin, and examined for light microscopically detectable alterations. Sites examined included the temporal cerebral cortex, caudate nucleus, internal capsule, lenticular nucleus, lateral ventricle, medial thalamus, hippocampus, third ventricle, midbrain, pons, cerebellum and medulla oblongata at levels of the middle cerebellar peduncle, obex, and rostral extremity of the spinal cord with the initial part of the central canal.

Additional sections of the medulla oblongata at the level of the obex were stained with immunohistochemical reagents. These sections were mounted on positively charged glass slides, deparaffinized, and hydrated. Pretreatments to enhance epitope exposure included immersion in 88% formic acid solution for 30 minutes followed by a rinse in water and then immersion in 10 μg/ml proteinase-K solution at 26 C for 10 minutes. This was followed by autoclaving for 20 minutes at 121 C in Tris buffer solution with cooling for 30 minutes. The sections were immunostained with an automated immunostainer[a] using PrP monoclonal antibody (MAb) F99/97.6.1 (provided by Katherine I. O'Rourke, US Department of Agriculture, ARS, Animal Disease Research Unit, Pullman, WA), a biotinylated secondary antibody, and alkaline phosphatase–streptavidin conjugate, a substrate chromogen, and a hematoxylin and bluing counterstain.[a] Monoclonal antibody F99/97.6.1 recognizes a conserved epitope on PrP and has been used to demonstrate PrP^{res} in CWD,[14] cattle inoculated intracerebrally with brain from CWD-affected cervids,[5] scrapie,[10] and BSE (D. H. Gould and E. Ehrensperger, unpublished data). Positive-control tissue from deer affected with CWD was included in each staining batch.

The number and age of cattle associated with particular locations in CWD-endemic regions were assessed. Game management units, which are geographical areas defined by the Colorado Division of Wildlife,[9] were used to define the endemic region. Cow-years in each game management unit were calculated to describe the time in contact with a CWD-contaminated environment with a certain estimated prevalence of disease.

The probability of a specific population being free of a disease can be determined from the exact hypergeometric distribution modified for imperfect tests[2] and can be easily calculated with the FreeCalc[b] computer program. Because the number of cattle selected from each herd was too small to distinguish disease-free herds from herds with a prevalence of less than 15%, freedom from disease could not be explored for individual herds. Hence, animals from the different herds were combined, and freedom from disease was assessed for the region encompassing the participating herds. This approach is only reasonable if clustering within herds does not occur. Further, because only culled cattle were tested, our results that were based on the assumption of random sampling are believed to be conservative. Finally, it was assumed that cattle affected with CWD have lesion features similar to those of BSE (and other TSEs) and that the test specificity and sensitivity using immunohistochemistry (IHC) for CWD in cattle are similar to those for other TSEs.

Table 1. Association of surveyed cattle with CWD-endemic areas.

Game management unit	Estimated CWD prevalence (%)*	No. of cows	No. of herds	Cow-years in a location
A	15	40	8	324.8
B	7	153	7	868.1
C	5	31	2	266
D	5	21	3	209.5
E	3	15	1	249
F	<5†	2	1	8.8
G	<5†	18	1	38.3

* Estimated prevalence in harvested mule deer; data are from Miller et al.[9] and M. W. Miller (unpublished data).

† Upper 95% confidence interval for estimated prevalence; CWD detected in game management unit by biased surveillance methods but not detected to date by random sampling of harvested deer.[9]

The minimum expected prevalence (MEP) is the minimum prevalence expected to be present if an area is affected by a disease. Because the MEP for CWD in cattle is unknown, the analysis was performed for a range of MEP levels. Because the IHC tests for CWD (T. R. Spraker, personal communication) and scrapie[c] are unlikely to produce false positives, our calculations were based on a test specificity of 100%. However, it is conceivable that infected animals were classified as disease free, especially if lesions had not yet appeared in the brain. Because only those animals most likely to have detectable disease were tested, it was believed that the test sensitivity was at least 90%, and calculations were performed for sensitivities of 90%, 95%, and 100%.

The brains of 262 culled cows from 22 herds were evaluated. The total population of these herds was 4,124. The mean age of the cows sampled was 8.2 years, the median age was 8 years, and the range was 4–16 years. The cows in our sample had spent an average of 7.5 years in their herd (median = 7 years, range = 4–16 years). Association of the cattle with game management units and estimated local CWD prevalence in deer is indicated in Table 1.

Microscopic examination of all 262 brains failed to reveal any indications of CWD or other TSE. Incidental findings in some brains included limited nonsuppurative (predominantly lymphocytic) meningoencephalitis, neuronal perikaryonic lipofuscin accumulation, occasional axonal spheroids in medullary nuclei, and mineralization of arteries and arterioles in the corpus striatum. Distinctly outlined, neuronal perikaryonic vacuoles were found in the red nucleus of many cows, and occasional neuronal perikaryonic vacuoles were found in other brainstem nuclei. Neuronal perikaryonic vacuoles were not associated with neuropil vacuolation, gliosis, or neuronal degeneration characterized by cell body shrinkage.

Immunohistochemically demonstrable PrP deposition was not evident in any of the brains.

The results of the tests for freedom from disease under the assumptions listed above provide the basis for limited conclusions. If the CWD prevalence in the study population is in the range of 2–10%, the probability of finding no diseased cattle in the sample of 262 cattle is 0 or near 0. Given an MEP of 0.05%, the probabilities of finding no diseased animals in a random sample of 262 out of 4,124 cattle were 25%, 27%, and 29% for sensitivities of 100%, 95%, and 90%, respectively. Assuming an MEP of 1%, the respective probabilities of finding no diseased animals in the sample were 7%, 8%, and 9%. Hence, assuming that the population of 4,124 cattle was infected and that the disease prevalence was 1% or less, the probability of finding no diseased animals in the sample was quite high. However, if the population of cattle was infected and the prevalence was 2% or more, the probability of finding no diseased animals in the sample was 0 or near 0 for all 3 sensitivity levels. These results indicate that only if the disease prevalence is high and the assumptions described above are satisfied can one conclude that the population of 4,124 cattle is most likely disease free. The results do not allow for conclusions about the other herds in the endemic region. If clustering within herds does occur, conclusions become more difficult to draw.

Although the actual exposure of the surveyed cows to the agent of CWD is unknown, the geographical locations and time spent in the locations indicate a higher risk of potential exposure than for other populations of cattle. Because the TSEs have long incubation periods, the advanced age of many of the cows increased the likelihood of detecting a TSE if it was present. Still, no histological or immunohistochemical features of a TSE were observed in any of the cows.

These findings are consistent with the results of other studies on the potential for transmission of CWD from cervids to cattle. All such studies that have been completed or are in progress indicate that the risk of CWD transmission to cattle is low relative to other animal TSEs. Studies of in vitro conversion of bovine PrP^{sen} to PrP^{res} by CWD PrP^{res} indicate low conversion efficiency compared with other prion pairs tested (scrapie and BSE).[11] Similarly, preliminary findings in a study involving intracerebral inoculation of cattle with CWD agent[5] demonstrate that CWD transmission to cattle is less efficient than scrapie transmission by the same exposure. Ongoing studies on cattle experimentally exposed to CWD by oral inoculation with tissue containing CWD agent during calfhood (E. S. Williams and M. W. Miller, unpublished data) or by prolonged contact with affected deer and contaminated premises (M. W. Miller and E. S. Williams, unpublished data) have failed to reveal evidence of disease in the cattle long after most concurrently exposed control deer succumbed to CWD. Understanding interspecies barriers to TSE transmission awaits further research. Such barriers are probably the result of a number of phenomena, including not only prion-conversion efficiency but also the nature of interactions between prions and cells of entry sites and the potential for prion translocation from entry sites to the central nervous system.

The occurrence of isolated, large perikaryonic vacuoles in brainstem neurons, especially those of the red nucleus, is observed as a nonspecific alteration in cattle.[4] These types of neuronal vacuoles are observed in the absence of other histological characteristics of TSEs, such as neuropil vacuolation, gliosis, neuronal degeneration, or PrP deposition.

Other histological alterations observed in the brains of these surveyed cattle are incidental findings and are unlike any of the features of the TSEs. Perikaryonic lipofuscin accumulation is an expected aging change in several neuronal populations of many animal species.[7] Likewise, the presence of spheroids in medullary nuclei is an expected age-associated change that reflects axonal degeneration. Mineralization of vessels of the corpus striatum has been reported in several species and does not appear to be associated with clinical disease.[15] The cause of the mild nonsuppurative encephalitis observed in some of the surveyed cattle is undetermined. Medulla oblongata brain tissues from the 20 cows with the most well-developed perivascular mononuclear cell infiltrations in the medulla were examined by polymerase chain reaction for nucleotide sequences of the viruses of epidemic hemorrhagic disease and bluetongue, bovine herpesvirus 1 and 5, and bovine virus diarrhea 1 and 2. All were negative.

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