University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Other Publications in Zoonotics and Wildlife Disease

Wildlife Disease and Zoonotics

2010

Increased risk of chronic wasting disease in Rocky Mountain elk associated with decreased magnesium and increased manganese in brain tissue

Stephen N. White
U.S. Department of Agriculture, Stephen.White@ARS.USDA.GOV

Katherine I. O'Rourke
U.S. Department of Agriculture, katherine.orourke@ars.usda.gov

Thomas Gidlewski
USDA-APHIS

Kurt C. VerCauteren
USDA-APHIS, kurt.c.vercauteren@aphis.usda.gov

Michelle R. Mousel
USDA-ARS

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/zoonoticspub

 Part of the Veterinary Infectious Diseases Commons

White, Stephen N.; O'Rourke, Katherine I.; Gidlewski, Thomas; VerCauteren, Kurt C.; Mousel, Michelle R.; Phillips, Gregory E.; and Spraker, Terry R., "Increased risk of chronic wasting disease in Rocky Mountain elk associated with decreased magnesium and increased manganese in brain tissue" (2010). *Other Publications in Zoonotics and Wildlife Disease*. 105.
https://digitalcommons.unl.edu/zoonoticspub/105

This Article is brought to you for free and open access by the Wildlife Disease and Zoonotics at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Other Publications in Zoonotics and Wildlife Disease by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors
Stephen N. White, Katherine I. O'Rourke, Thomas Gidlewski, Kurt C. VerCauteren, Michelle R. Mousel, Gregory E. Phillips, and Terry R. Spraker

This article is available at DigitalCommons@University of Nebraska - Lincoln: https://digitalcommons.unl.edu/zoonoticspub/105

Increased risk of chronic wasting disease in Rocky Mountain elk associated with decreased magnesium and increased manganese in brain tissue

Stephen N. White, Katherine I. O'Rourke, Thomas Gidlewski, Kurt C. VerCauteren, Michelle R. Mousel, Gregory E. Phillips, Terry R. Spraker

Abstract

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) of Rocky Mountain elk in North America. Recent studies suggest that tissue and blood mineral levels may be valuable in assessing TSE infection in sheep and cattle. The objectives of this study were to examine baseline levels of copper, manganese, magnesium, zinc, selenium, and molybdenum in the brains of Rocky Mountain elk with differing prion genotypes and to assess the association of mineral levels with CWD infection. Elk with leucine at prion position 132 had significantly lower magnesium levels than elk with 2 copies of methionine. Chronic wasting disease-positive elk had significantly lower magnesium than control elk. The incorporation of manganese levels in addition to magnesium significantly refined explanatory ability, even though manganese alone was not significantly associated with CWD. This study demonstrated that mineral analysis may provide an additional disease correlate for assessing CWD risk, particularly in conjunction with genotype.

Résumé

La maladie du dépérissement chronique (CWD) est une encéphalopathie spongiforme transmissible (TSE) des wapitis en Amérique du Nord. Des études récentes suggèrent que la mesure des taux de minéraux dans les tissus et le sang seraient utiles pour évaluer une infection par TSE chez les moutons et les bovins. Les objectifs de la présente étude étaient d'examiner les niveaux de base de cuivre, manganèse, magnésium, zinc, sélénium et molybdène dans le cerveau de wapitis avec des génotypes différents de sensibilité envers les maladies à prions et d'évaluer l'association des niveaux de minéraux avec l'infection CWD. Les wapitis avec une leucine à la position 132 du prion présentaient des niveaux significativement plus faibles de magnésium que les wapitis avec deux copies de méthionine. Les wapitis positifs pour CWD avaient des niveaux de magnésium significativement plus faibles que les wapitis témoins. L'ajout des niveaux de manganèse à ceux du magnésium a permis de raffiner significativement la capacité d'explication, même si le manganèse seul n'était pas associé significativement avec le CWD. Cette étude démontre que l'analyse des minéraux peut fournir un corrélat additionnel pour évaluer le risque de CWD, particulièrement en conjonction avec le génotype.

(Traduit par Docteur Serge Messier)

Chronic wasting disease (CWD) is the transmissible spongiform encephalopathy (TSE) of North American deer, elk, and moose (1). The TSEs are fatal neurodegenerative disorders thought to be caused by an infectious prion agent composed of a misfolded variant (PrP^d) of the normally occurring host cellular prion protein (PrP^c) (2). Misfolding of the host PrP^c by exogenous or sporadically misfolded PrP^d initiates the disease process (2). Control of CWD in elk in the United States (US) and Canada includes depopulation or permanent quarantine of infected farmed herds and culling of free-ranging suspects showing clinical signs. Despite these efforts, new outbreaks are reported in both the US and Canada (3,4) and the economic losses to the industry and regulatory bodies are substantial. The management of herds with a small number of animals introduced from infected farms is a concern. Introduction of an animal from an infected farm is a risk factor for CWD in animals housed together, with substantial increase in risk if the introduced animal developed clinical disease (4). An additional risk factor includes the normal prion protein precursor (PRNP) genotype, with a lower risk of disease in some populations for elk homozygous or heterozygous for the version of the PRNP gene which encodes a leucine residue at position 132 (132L) (5,6).

Levels of tissue minerals known to bind PrP^c are associated with TSEs in several model systems. The association between TSE and copper (Cu) or manganese (Mn) has been reported (7,8); both of these minerals bind PrP^c and may affect the efficiency of protein misfolding (9). Elevated Mn levels in brain and blood have been reported with bovine, ovine, and human TSEs (7,8,10). Additional divalent cations of appropriate size and ionic strength may also have roles in TSEs,

USDA-ARS Animal Disease Unit, Pullman, Washington, USA (White, O'Rourke); Washington State University College of Veterinary Medicine, Pullman, Washington, USA (White, O'Rourke); USDA-APHIS, Veterinary Services (Gidlewski), and USDA-APHIS, Wildlife Services (VerCauteren, Phillips), National Wildlife Research Center, Fort Collins, Colorado, USA; USDA-ARS US Sheep Experiment Station, Dubois, Idaho, USA (Mousel); Colorado State University College of Veterinary Medicine & Biomedical Sciences, Fort Collins, Colorado, USA (Spraker).

Address all correspondence to Dr. Stephen White; telephone: (509) 335-7407; fax: (509) 335-8328; e-mail: swhite@vetmed.wsu.edu

Dr. White's current address is Animal Disease Research Unit, PO Box 646630, Pullman, Washington 99164, USA.

Received April 17, 2008. Accepted December 15, 2008.

This article is a U.S. government work, and is not subject to copyright in the United States.

although it is not known whether these mineral level changes reflect a predisposing influence on prion protein folding or a downstream effect of the disease processes (11). Baseline data on mineral levels in the brain of Rocky Mountain elk *(Cervus elaphus nelsoni)* are not available, and the confounding effects of varying feed sources and PRNP genotypes are not known. The objectives of this study were to provide baseline data on mineral levels in brain tissue from elk collected during regulatory depopulation of infected herds, to estimate mineral levels in elk of different genotypes without evidence of disease, and to explore potential associations between tissue mineral concentrations and presence of CWD in captive Rocky Mountain elk.

Tissue samples were collected from 223 Rocky Mountain elk that were euthanized as part of a joint federal and state control program. Elk originated from 3 United States farms, each of which had multiple confirmed cases of CWD. Brains, tonsils, retropharyngeal lymph nodes and sometimes liver were collected at necropsy. DNA was extracted from frozen liver or brain and the open reading frame of the gene for PRNP was amplified and sequenced (5). Genotypes at amino acid position 132 were recorded as 132MM (2 copies of methionine) or 132Lx (at least one copy of leucine) (5). Brain, retropharyngeal lymph node, and tonsil were formalin fixed and processed for detection of PrP^d by an automated monoclonal antibody immunohistochemistry assay (12). Animals were considered CWD positive if at least one tissue was positive for PrP^d. Because of the unknown lag between infection and appearance of detectable PrP^d (4), elk exposed to CWD but lacking detectable PrP^d were not defined as CWD negative; these animals were defined as herd-matched controls.

Trace minerals (Cu, Mn, Mg, Zn, Se, and Mo) were analyzed in samples from the cerebrum, parietal lobe, or optic lobe of the brain collected at necropsy. For Cu, Mn, and Zn analyses, the tissues were dried, weighed, ashed overnight in a muffle furnace, and then dissolved in dilute HNO_3. The solutions were then analyzed by flame atomic absorption spectrophotometry (FAAS) (13). Magnesium was measured with a slight modification: a releasing agent (lanthanum oxide in dilute HCl) was added to the HNO_3 solutions prior to FAAS (13). For Se, the tissues were dried, weighed, and then digested with $Mg(NO_3)_2$ and concentrated HNO_3 overnight on a hot plate under a hood. The digests were ashed in a pre-heated muffle furnace, the cooled ashes were dissolved in dilute HCl, and the solutions were analyzed by Hydride Generation FAAS (14,15). For Mo, the tissues were dried, weighed, ashed overnight in a muffle furnace and then dissolved in dilute HNO_3. Palladium nitrate [$Pd(NO_3)_2$] and ascorbic acid matrix modifers were added to the solutions, and the solutions were analyzed by Graphite Furnace Atomic Absorption Spectrophotometry (16). All concentrations were reported in μg/g (dry mass basis), and standard quality control samples spiked with the metal under investigation were included with each analysis.

The relationships between CWD status, PRNP genotype, and mineral levels in the brain were evaluated using a sample of 223 Rocky Mountain elk from 3 US facilities with similar CWD prevalence. These animals were divided into 3 groups based on genotype and CWD status, including 132Lx genotype control elk (n = 50), 132MM control elk (n = 146), and 132MM CWD elk (n = 27). Because no 132Lx animals were positive in this sample, comparisons between 132Lx control elk (n = 50) and 132MM control animals (n = 146) were used to compare mineral levels of 132MM and 132Lx elk without detectable PrP^d. Genotypic differences in cation levels in the brain from control elk were examined by using t-tests to compare samples from elk with the PRNP 132MM genotype to samples from elk with the 132Lx genotype. Because the samples failed preliminary tests for equal variance, Satterthwaite's method was used to estimate degrees of freedom for t-tests.

All CWD positive elk in this study (n = 27) had 132MM genotypes; therefore, only 132MM samples (n = 146 samples with no detectable PrP^d) were used as controls for CWD investigation. Samples from the CWD positive and the genotype-matched control elk were examined for differing cation levels and the results were evaluated using a t-test (SAS Institute, Cary, North Carolina, USA). Logistic regression was used to explore potential relationships between CWD status (response variable) and explanatory variables sex, location, age at death (in years), and mineral concentrations (μg/g) of Cu, Mg, Mn, Mo, Se, and Zn in the brain (logistic procedure in SAS). This analysis used an exploratory approach, and stepwise automated model selection procedures of the SAS logistic procedure were used to evaluate models including up to 9 variables and their interactions. Nagelkerke's adjusted coefficient of determination was used to assess the fraction of variation explained by the resulting model. Incremental change in mineral concentrations associated with doubling of the odds of detecting CWD (odds ratio = 2) were used to interpret the final model, where each mineral concentration was varied while holding others constant at the mean level.

Baseline mineral data for CWD control elk are shown by genotype in Table 1. Brain from control elk with the 132Lx long incubation genotype had significantly lower mean Mg than brain from 132MM control elk (346.1 μg/g versus 412.2 μg/g; t = 3.17, 106 df; P = 0.002; Table I). None of the other minerals differed by genotype ($P > 0.05$; Table I).

Magnesium levels were lower (314.1 μg/g versus 412.2 μg/g; t = 3.12, 36.1 df; P = 0.004) in CWD positive elk but other minerals did not differ by CWD status (Table II). Logistic regression models were used to better explain variation in CWD status using multiple explanatory variables. There was little evidence of any relationship between CWD status and sex, location, age, and the cations Cu, Mo, Se, Zn ($P > 0.05$). The final model included levels of Mg (Wald chi-square = 16.3, 1 df; $P < 0.0001$) and Mn (Wald chi-square = 10.7, 1 df; P = 0.001) in the brain. Combined use of Mn and Mg explained 21% of variation in CWD status (R^2 = 0.21) compared with 10% using Mg alone (R^2 = 0.10) indicating the value of both metals in supplying complementary information. Table III shows increments of each cation required to double odds of detecting CWD, given the final model and cation levels measured from brain tissue. Each decrease of 85 μg/g Mg doubled the odds of detecting CWD, if Mn levels were held constant. Similarly, each increase of 0.48 μg/g Mn doubled the odds of detecting CWD, if Mg levels were held constant.

The effect of PRNP genotype on baseline Mg was significant in this study; control elk with the 132MM short incubation genotype had higher Mg levels than elk with the 132Lx long incubation genotype from the same facilities. Since Mg plays a critical role in membrane stability, energy balance, oxidative stress, and neurotransmitter release following brain injury (17), the effect of genotype-related differences in baseline Mg may be of interest in understanding the

Table I. Metal concentrations in the brain and their respective standard errors from Rocky Mountain elk with 132MM and 132Lx genotypes in the absence of detectable PrP^d (CWD control elk)

Elk genotype and CWD status	Mg	Mn	Cu	Mo	Zn	Se
132MM-control	412.2	1.32	12.1	0.33	39.4	0.55
n = 146	(12.3)	(0.05)	(.40)	(0.02)	(1.3)	(0.01)
132Lx-control	346.1[a]	1.15	11.6	0.25	36.7	0.51
n = 50	(16.8)	(0.11)	(0.58)	(0.02)	(2.2)	(0.02)

Mg — magnesium; Mn — manganese; Cu — copper; Mo — molybdenum; Zn — zinc; Se — selenium.

[a] Different from the 132MM control elk ($P = 0.002$).

Table II. Metal concentrations in the brain and their respective standard errors from CWD-positive and control Rocky Mountain elk with the 132MM genotype

Elk genotype and CWD status	Mg	Mn	Cu	Mo	Zn	Se
132MM-CWD	314.1[a]	1.5	12.6	0.30	36.7	0.57
n = 27	(29.0)	(.011)	(0.86)	(0.03)	(3.3)	(0.03)
132MM-control	412.2	1.32	12.1	0.33	39.4	0.55
n = 146	(12.3)	(0.05)	(.40)	(0.02)	(1.3)	(0.01)

Mg — magnesium; Mn — manganese; Cu — copper; Mo — molybdenum; Zn — zinc; Se — selenium.

[a] Different from the 132MM control elk ($P = 0.004$).

Table III. Increments of magnesium and manganese associated with doubling the odds of detecting CWD, given the final model and cation levels measured from brain tissue

Brain cation	Odds ratio	95% CI	Change in cation concentration (μg/g)
Mg	2.00	– 0.35, – 0.69	– 85.0
Mn	2.00	1.34, 3.12	0.48

Mg — magnesium; Mn — manganese.

CI — confidence interval.

pathogenesis of long incubation TSE phenotypes. Magnesium levels in ruminant livestock are tightly regulated within a range controlled by diet, undefined genetic elements, and reproductive status, particularly in association with lactation (18). Although standards for Mg and other minerals in brain from Rocky Mountain elk are not reported, the levels in this study (Tables I–II) were similar to those reported for liver mineral levels from Tule elk *(Cervus elaphus nannodes)* (19). Consideration of environmental and forage factors in association with PRNP genotype in study populations will be useful in establishing baseline data on mineral levels in captive Rocky Mountain elk.

When only elk with the 132MM short incubation genotype were considered, lower Mg levels were observed in brain from CWD positive elk than in brain from control elk from the same herds. This was true despite the possibility that some of the control animals could have been very early stage positives, which would have made it more difficult to establish significant differences between groups. The finding of reduced Mg in brain from elk with natural CWD is consistent with a similar finding in mice experimentally infected with scrapie (20), which suggests that Mg reduction may be a feature of many TSEs. Reduced magnesium is an almost ubiquitous feature of central nervous system injury (17), however, and the effect is not likely to be specific to the TSEs.

Elevated levels of Mn in the peripheral blood may be useful as an early indicator of TSE infection (7,10). Because disease-causing PrP^d accumulates most heavily in nervous tissue and especially the brain (12), this study examined brain tissue levels of Mn. Although brain tissue from CWD-positive elk had a trend toward elevated Mn, the relationship was weak. This finding is consistent with a recent report on Mn levels in the central nervous system of sheep, in which Mn levels in infected sheep rose within a month following infection (7). PrP^c binds Mn at 2 sites, both of which are upstream from the residue 132 substitution, so a direct effect of the mutation on Mn binding is not predicted (9).

However, the combined model using Mn while accounting for Mg reveals the potential importance of Mn by quantifying the relationship of Mg and Mn with detection of CWD under the conditions that existed in this study. Nagelkerke's adjusted R^2 suggests that the model with both Mn and Mg explained approximately twice as much variation in CWD status as the use of Mg alone. Changes in concentration well within the range of observed values for either Mg or Mn were associated with a doubling of the odds of CWD. Although this study detected 2 risk-associated minerals for elk of the 132MM genotype, it does not establish a causal link between Mg and Mn concentrations and CWD. However, if absolute levels or changes in tissue Mg and Mn concentrations are symptomatic of CWD, they may have practical application for herd surveillance if additional factors are identified to improve diagnostic precision of models. Because Mg levels differed between genotypes of control elk and because 132MM-CWD elk had similar Mg levels as 132Lx-control elk, it will be important to consider genotype in future studies.

Chronic wasting disease in captive elk is controlled largely by whole herd depopulation. However, the cost of indemnity payments, loss of animal life, and hardship to producers is considerable

and there is no assurance that the disease will not recur following restocking of potentially contaminated facilities (1). Mineral-based approaches to TSE diagnosis and pathogenesis have been proposed but data on baseline levels in captive Rocky Mountain elk is lacking. We report PRNP associated differences in Mg levels in the elk in this study. When PRNP genotype was held constant, Mg and Mn concentrations in brain tissue were related to CWD status in a logistic regression model. If the present results can be replicated with blood or some other easily accessible tissue, understanding of relative risk and disease course for individual animals might be enhanced by studies measuring changes in mineral levels after experimental inoculation with CWD. This study demonstrated potential for using multi-mineral predictors for detecting CWD and the need for additional predictors to enhance diagnostic precision.

Acknowledgments

The authors gratefully acknowledge Codie Hanke for technical assistance, Cathy Bedwell for mineral analysis, veterinarians and technicians with the USDA Animal Plant Health Inspection Service and the state of Colorado for epidemiology and tissue collection, and Jennifer Swenson and Elaine Anderson for meticulous record keeping. Supported by grants from the United States Department of Agriculture, Agricultural Research Service 5348-32000-026-00D, Specific Cooperative Agreement 58-5348-2-678, CSREES 2003-51140-02126, and the College of Veterinary Medicine, Colorado State University.

References

1. Williams ES. Chronic wasting disease. Vet Path 2005;42:530–549.
2. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982;216:136–144.
3. Miller MW, Williams ES, McCarty CW, et al. Epizootiology of chronic wasting disease in free-ranging cervids in Colorado and Wyoming. J Wildl Dis 2000;36:676–690.
4. Argue CK, Ribble C, Lees VW, McLane J, Balachandran A. Epidemiology of an outbreak of chronic wasting disease on elk farms in Saskatchewan. Can Vet J 2008;48:1241–1248.
5. O'Rourke KI, Besser TE, Miller MW, et al. PrP genotypes of captive and free-ranging Rocky Mountain elk *(Cervus elaphus nelsoni)* with chronic wasting disease. J Gen Virol 1999;80:2765–2769.
6. Perucchini M, Griffin K, Miller MW, Goldmann W. PrP genotypes of free-ranging wapiti *(Cervus elaphus nelsoni)* with chronic wasting disease. J Gen Virol 2008;89:1324–1328.
7. Hesketh S, Sassoon J, Knight R, Hopkins J, Brown DR. Elevated manganese levels in blood and central nervous system occur before onset of clinical signs in scrapie and bovine spongiform encephalopathy. J Anim Sci 2007;85:1596–1609.
8. Thackray AM, Knight R, Haswell SJ, Bujdoso R, Brown DR. Metal imbalance and compromised antioxidant function are early changes in prion disease. Biochem J 2002;362:253–258.
9. Brazier MW, Davies P, Player E, Marken F, Viles JH, Brown DR. Manganese binding to the prion protein. J Biol Chem 2008;283:12831–12839.
10. Hesketh S, Sassoon J, Knight R, Brown DR. Elevated manganese levels in blood and CNS in human prion disease. Mol Cell Neurosci 2008;37:590–598.
11. Choi CC, Kanthasamy A, Anantharam V, Kanthasamy AG. Interaction of metals with prion protein: Possible role of divalent cations in the pathogenesis of prion diseases. Neurotoxicology 2006;27:777–787.
12. Spraker TR, Balachandran A, Zhuang D, O'Rourke KI. Variable patterns of PrP-CWD distribution in obex and cranial lymphoid tissues of Rocky Mountain elk with nonclinical chronic wasting disease. Vet Rec 2004;155:295–302.
13. Helrich HE. Official methods of analysis of the Association of Official Analytical Chemists. Gaithersburg, Maryland: AOAC, 1990:Method 974,27.
14. Stahr HM. Analytical Methods in Toxicology. New York, New York: John Wiley & Sons, 1991.
15. Poole CF, Evans NJ, Wibberly DG. Determination of selenium in biological samples by gas-liquid chromatography with electron-capture detection. J Chromatogr 1977;136:73–83.
16. Tracy M, Melton L, Holstege D. Molybdenum quantitation by GFZAA. In: CVDLS Toxicology Laboratory Standard Methods. Davis, California: University of California, 1996.
17. Vink R, Cernak I. Regulation of intracellular free magnesium in central nervous system injury. Front Biosci 2000;5:D656–D665.
18. Goff JP. Macromineral disorders of the transition cow. Vet Clinic NA — Food Animal Practice 2004;20:471–494.
19. Johnson HE, Bleich VC, Krausman PR. Mineral deficiencies in tule elk, Owens Valley, California. J Wildl Dis 2007;43:61–74.
20. Wong BS, Brown DR, Pan T, et al. Oxidative impairment in scrapie-infected mice is associated with brain metals perturbations and altered antioxidant activities. J Neurochem 2001;79:689–698.