PRION PROTEIN GENES IN CARIBOU FROM ALASKA

Authors: Happ, George M., Huson, Heather J., Beckmen, Kimberlee B., and Kennedy, Lorna J.

Source: Journal of Wildlife Diseases, 43(2) : 224-228

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-43.2.224

Journal of Wildlife Diseases, 43(2), 2007, pp. 224–228

PRION PROTEIN GENES IN CARIBOU FROM ALASKA

George M. Happ,[1,4] Heather J. Huson,[1] Kimberlee B. Beckmen,[2] and Lorna J. Kennedy[3]

[1] Institute of Arctic Biology, University of Alaska Fairbanks, PO Box 757000, Fairbanks, Alaska 99775-7000, USA
[2] Alaska Department of Fish and Game, 1300 College Road, Fairbanks, Alaska, 99701-1599, USA
[3] Centre for Integrated Genomic Medical Research, University Manchester, Stopford Building, Oxford Road, Manchester, M13 9PT, UK
[4] Corresponding author (email: ffgmh@uaf.edu)

ABSTRACT: Prion protein genes were sequenced in free-ranging Alaska caribou (*Rangifer tarandus grantii*). Caribou prion alleles are identical or nearly so to those of wapiti, white-tailed deer, and mule deer. Five single-nucleotide polymorphisms were detected with substitutions at residues 2 (V→M), 129 (G→S), 138 (S→N), 146 (N→N), and 169 (V→M). The 138N codon had been previously reported only in prion pseudogenes of other cervids. In caribou, the 138S and 138N alleles are present at frequencies of approximately 0.7 and 0.3, respectively, and they are seen in both homozygotes and heterozygotes of three geographically separated herds, each a component of the continental metapopulation. Genetics seems to permit the spread of chronic wasting disease from middle-latitude deer to high-latitude caribou in North America.

Key words: Chronic wasting disease, caribou, prion, *Rangifer tarandus grantii*, reindeer, transmissible spongiform encephalopathy.

INTRODUCTION

Prion proteins (PrP) are thought to be the agents responsible for neurodegenerative spongiform encephalopathies (Prusiner, 1998). The most studied prion disease of wild animals is chronic wasting disease (CWD) of mule deer (*Odocoileus hemionus hemionus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*) (Williams and Miller, 2002). Chronic wasting disease was noticed in 1967 as a clinical syndrome in captive deer and then recognized as a spongiform encephalopathy in 1978 (Williams and Young, 1980). Since its original appearance in Colorado and southern Wyoming, CWD has been diagnosed in free-ranging and farmed deer and elk over an increasingly wider geographical area (Belay et al., 2004).

Chronic wasting disease has not been found yet in caribou, but the natural range of caribou overlaps those of CWD-susceptible deer in Canada and Alaska. Polymorphisms of the gene (*PRNP*) encoding the prion proteins of deer have been linked to disease susceptibility (O'Rourke et al., 2004; Johnson et al., 2006). Susceptibility of caribou to prion infections could be strongly influenced by the sequences of the caribou prion genes. Because caribou are major components of subsistence diets of rural peoples in northern Alaska, Canada, and Eurasia, sensitivity to potential discovery of CWD in caribou is high.

Caribou comprise a metapopulation, divisible into herds that vary in size from scores of individuals to hundreds of thousands. Thirty such herds exist in Alaska. We report the frequencies for five *PRNP* alleles and the prion genotypes for caribou from the Porcupine, Delta, and Western Arctic herds in Alaska.

MATERIALS AND METHODS

Biological materials

Blood was collected from caribou (*Rangifer tarandus grantii*) of three herds. The nonmigratory Delta herd (4,500 animals) is restricted to interior Alaska, whereas both the Porcupine herd (130,000 animals) and the Western Arctic herd (450,000 animals) migrate between summer calving grounds near the Arctic Ocean to wintering grounds south of the Brooks Range (Fig. 1).

Polymerase chain reaction (PCR) and cycle sequencing

Blood cells were lysed with Qiagen protease (QIAGEN, Valencia, California, USA), and genomic DNA was extracted using the QIAGEN QIAamp kit (QIAGEN). The open

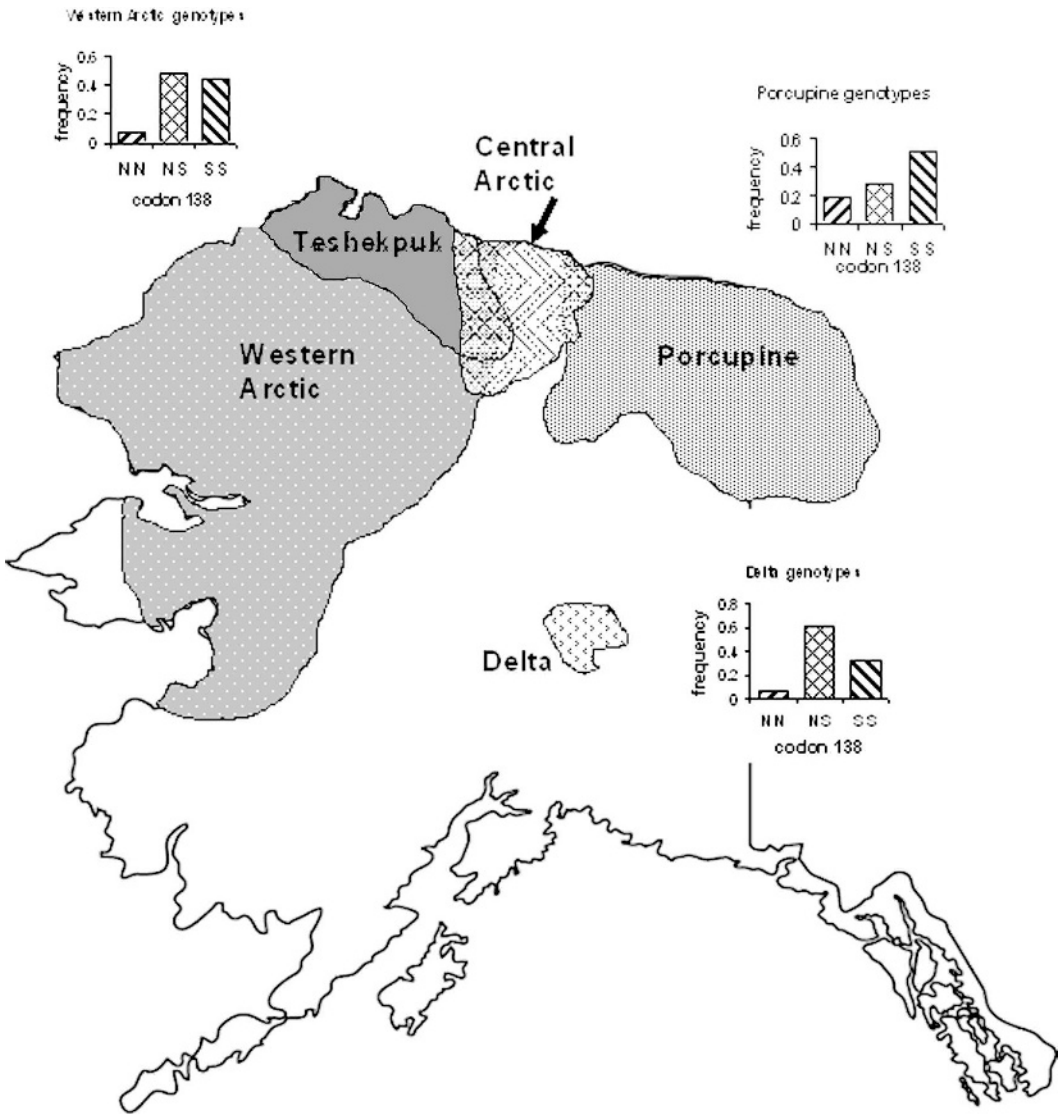

FIGURE 1. Distribution of five caribou herds in Alaska. The frequencies of serine (S) and asparagine (N) genotypes for codon are shown in the bar graphs for Western Arctic, Porcupine, and Delta herds.

reading frame of the third exon of the *PRNP* gene was amplified in a protocol modified after O'Rourke et al. (1999). Conditions for the PCR reaction on Porcupine samples were 5 µl of 10× PCR buffer, 4–5 µl of 2.5 mM each dNTP, 4–4.5 µl of 25 mM $MgCl_2$, 2.5–5 µl of 10 pmol/µl forward and reverse primers, 0.25 µl of 5 U/µl of DNA polymerase, and 5 µl of genomic DNA in a 50-µl reaction. Forward primers were either Ce19-ATTTTGCAGATAAGTCATC or Ce19v2-CTTTATTTTGCAGATAAGTC, and the reverse primer was Ce778-AGAAGATAATGAAAACAGGAAG. For Porcupine, we used the Ce19 primers and ABI AmpliTaq polymerase (Applied Biosystems, Foster City, California, USA) at 94 C for 4 min, and then 40 cycles at 94 C for 30 sec, 50.5 C for 30 sec, and 72 C for 30 sec, followed by 72 C for 10 min. For Delta and Western Arctic samples, the Ce19v2 forward primer was used in a QIAGEN Hot StarTaq protocol of 95 C for 15 min and then 35–40 cycles of 94 C for 30 sec, 51.5 C for 30 sec, and 72 C for 1 min, with a final extension of 72 C for 10 min. Porcupine samples were rese-

TABLE 1. Prion proteins in caribou herds in Alaska. Deduced amino acids are shown for the nonsynonymous substitutions at codons 2, 129, 138, and 169. In the Delta herd, three animals showed a synonymous substitution at base pair 438 and coded for VGSV alleles.

Protein	Delta herd $n=37$	Porcupine herd $n=31$	Western Arctic herd $n=40$
VGNV	22	18	24
VGSV	46	40	53
VSSV	0	3	1
MSSM	6	1	2
Total no. of alleles scored	74	62	80

quenced using the Hot StarTaq protocol as a positive control. To detect a prion pseudogene like that of mule deer, we used primer set 369/224 of Brayton et al. (2004). Polymerase chain reaction products from 31 Porcupine, 40 Western Arctic, and 37 Delta caribou were sequenced using ABI Big Dye and 3100 Genetic Analyzer (GenBank accession nos. DQ154292–DQ154296).

RESULTS

The open reading frame (771 nucleotides) in the caribou *PRNP* encodes a primary translation product of 256 amino acids. Cleavage of the N-terminal signal sequence should yield a protein of 232 amino acids (Gossert et al., 2005). Single-nucleotide polymorphisms (SNPs) occur at bases 4 (codon 2, V/M), 385 (codon 129, G/S), 413 (codon 138, S/N), 438 (codon 146, N/N), and 505 (codon 169, V/M). No pseudogene was detected with the primers of Brayton et al (2004).

Five alleles encode four different proteins in the three herds. Four alleles were found as homozygotes. The fifth allele with thymine at base 438 is synonymous with the predominant 438C and was seen in three heterozygotes of the Delta herd. The SNP in codon 2 (V2M) lies in the putative signal sequence and thus has no impact on the conformation of the mature protein. Alleles encoding proteins containing VGNV, VGSV, and MSSM were detected in all three herds (Table 1).

The genotypic frequencies for S138N alleles for all caribou sequenced (SS 0.10, SN 0.47, and NN 0.43) were not significantly different from Hardy-Weinberg equilibrium values (chi-square test, $\chi^2=2.10$, $P=0.59$), suggesting that the serine/asparagine polymorphism is not under strong selection across the metapopulation. The genotypes for codon 138 in each herd are shown in Figure 1. Within our sample of each herd, there was no significant departure from Hardy-Weinberg equilibria (Fisher exact probability test). Pairwise comparisons showed no significant differences between Western Arctic and the other two herds, but the Porcupine and Delta herds were significantly different by the Fisher exact probability test ($P=0.039$). This result could be an artifact due to small sample sizes.

DISCUSSION

Could any of the SNPs we report in caribou *PRNP* influence transmission of CWD? Chronic wasting disease is known in three species of cervids with similar or identical PrP genes (Heaton et al., 2003; Johnson et al., 2003). Efficient transmission requires a close conformational fit between the invasive PrP^{sc} and the PrP^{c} of the host (Raymond et al., 2000; Caughey, 2003). The sequence of the most common caribou allele (GenBank accession no. DQ154293; VGSV in Table 1) is identical to a mule deer allele (GenBank accession no. AY228473). The replacement of codon 138 serine with asparagine, seen in almost half of our caribou samples from Alaska and in all three herds, has been detected previously in pseudogenes of white-tailed deer (O'Rourke et al., 2004) and mule deer (Brayton et al., 2004). Polymorphisms at codon 138 affect the folding of cellular PrP^{c} to the pathological isoform PrP^{sc}. In cell-free reactions, purified cervid S138 PrP^{sc} proteins were more efficient at converting S138 PrP^{c} than N138 PrP^{c} to PrP^{sc} (Raymond

et al., 2000). The apparent excess of S138N heterozygotes in the Delta herd (Fig. 1) could confer some protection from CWD for this herd, which is more likely to make contact with mule deer wandering from Canada and with elk farmed in that region of Alaska. In addition, the SNP specifying methoinine at codon 169, which is found most commonly in the Delta herd, might perturb the protein loop domain created by residues 169–178 (Gossert et al., 2005) and thus influence susceptibility to CWD.

Transmission of prion disease from cervids to other mammals has not been documented in nature. No transmission of CWD from deer to cattle was detected when deer and cattle shared the same pasture (Gould et al., 2003). Isolated case reports that suggested a causative link between human neurological disease and consumption of wild deer (Belay et al., 2001) have not survived rigorous epidemiological scrutiny (Belay et al., 2004). However, intracerebral injections of brain homogenates from CWD-afflicted mule deer can produce pathology in cattle (Hamir et al., 2005). Hunters are often cautioned about butchering and consumption of deer in localities of epizootic CWD (Belay and Schonberger, 2005). Surveillance of wild and captive cervids in Alaska and Canada seems prudent.

Because they inhabit high-latitude sites remote from human population centers, each herd of caribou forms a relatively pristine unit that has been minimally perturbed by human interventions. Although the herds we examined are allopatric, gene flow between them is possible through intermediate herds (Fig. 1). Analysis of SNPs at prion loci of caribou from Alaska might offer opportunities to determine whether allelic and genotypic frequencies carry an unambiguous signature of balancing selection. The field-collected data could complement experiments with purified proteins (Raymond et al., 2000) and with "cervidized" mice (Browning et al., 2004) as well as with controlled infections in captive cervids.

ACKNOWLEDGMENTS

We thank James Dau, Mark Keech, and Ken Whitten for collecting blood and Amanda Read for comments on the manuscript. This project was supported in part by the National Science Foundation grant 0346770 and the National Institutes of Health grant 2 P20 157 RR016466.

LITERATURE CITED

Belay, E. D., and L. B. Schonberger. 2005. The public impact of prion diseases. Annual Review of Public Health 26: 191–212.

———, P. Gambetti, L. B. Schonberger, P. Parchi, D. R. Lyon, S. Capellari, J. H. McQuiston, K. Bradley, G. Dowdle, J. M. Crutcher, and C. R. Nichols. 2001. Creutzfeldt-Jakob disease in unusually young patients who consumed venison. Archives of Neurology 58: 1673–1678.

———, R. A. Maddox, E. S. Williams, M. W. Miller, P. Gambetti, and L. B. Schonberger. 2004. Chronic wasting disease and potential transmission to humans. Emerging Infectious Diseases 10: 977–984.

Brayton, K. A., K. I. O'Rourke, A. K. Lyda, M. W. Miller, and D. P. Knowles, Jr. 2004. A processed pseudogene contributes to apparent mule deer prion gene heterogeneity. Gene 326: 167–173.

Browning, S. R., G. L. Mason, T. Seward, M. Green, G. A. Eilason, C. Mathiason, M. W. Miller, E. S. Williams, E. Hoover, and G. C. Telling. 2004. Transmission of prions from mule deer and elk with chronic wasting disease to transgenic mice expressing cervid PrP. Journal of Virology 78: 13345–13350.

Caughey, B. 2003. Prion protein conversions: Insight into mechanisms, TSE transmission barriers and strains. British Medical Journal 66: 109–120.

Gossert, A. D., S. Bonjour, D. A. Lysek, F. Fiorito, and K. Wüthrich. 2005. Prion protein NMR structures of elk and mouse/elk hybrids. Proceedings of the National Academy of Sciences of the United States of America 102: 646–650.

Gould, D. H., J. L. Voss, M. W. Miller, A. M. Bachand, B. A. Cummings, and A. A. Frank. 2003. Survey of cattle in northeast Colorado for evidence of chronic wasting disease: Geographical and high-risk targeted sample. Journal of Veterinary Diagnostic Investigation 15: 274–277.

Hamir, A. N., R. A. Kunkle, R. C. Cutlip, J. M. Miller, K. I. O'Rourke, E. S. Williams, M. W. Miller, M. J. Stack, M. J. Chaplin, and J. A. Richt. 2005. Experimental transmission of chronic wasting disease agent from mule deer

to cattle by the intercerebral route. Journal of Veterinary Diagnostic Investigation 17: 276–281.

HEATON, M. P., K. A. LEYMASTER, B. A. FREKING, D. A. HAWK, T. P. SMITH, J. W. KEELE, W. M. SNELLING, J. M. FOX, C. G. CHITKO-MCKOWN, AND W. W. LAEGREID. 2003. Prion gene sequence variation within diverse groups of U.S. sheep, beef cattle, and deer. Mammalian Genome 14: 765–777.

JOHNSON, C., J. JOHNSON, M. CLAYTON, D. MCKENZIE, AND J. AIKEN. 2003. Prion protein heterogeneity in free-ranging white-tailed deer within the chronic wasting disease affected region of Wisconsin. Journal of Wildlife Diseases 39: 576–581.

———, ———, J. P. VANDERLOO, D. KEANE, J. M. AIKEN, AND D. MCKENZIE. 2006. Prion protein polymorphisms in white-tailed deer influence susceptibility to chronic wasting disease. Journal of General Virology 87: 2109–2114.

O'ROURKE, K. I., T. E. BESSER, M. W. MILLER, T. F. CLINE, T. R. SPRAKER, A. L. JENNY, M. A. WILD, G. L. ZEBARTH, AND E. S. WILLIAMS. 1999. PrP genotypes of captive and free-ranging Rocky Mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease. Journal of General Virology 80: 2765–2769.

———, T. R. SPRAKER, L. K. HAMBURG, T. E. BESSER, K. A. BRAYTON, AND D. P. KNOWLES. 2004. Polymorphisms in prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer. Journal of General Virology 85: 1339–1346.

PRUSINER, S. B. 1998. Prions. Proceedings of the National Academy of Sciences of the United States of America 95: 13363–13383.

RAYMOND, G. J., A. BOSSERS, L. D. RAYMOND, K. I. O'ROURKE, L. E. MCHOLLAND, P. K. BRYANT 3RD, M. W. MILLER, E. S. WILLIAMS, M. SMITS, AND B. CAUGHEY. 2000. Evidence of a molecular barrier limiting susceptibility of humans, cattle, and sheep to chronic wasting disease. EMBO Journal 19: 4425–4430.

WILLIAMS, E. S., AND M. W. MILLER. 2003. Transmissible spongiform encephalopathies in non-domestic animals: Origin, transmission and risk factors. Revue Scientifique et Technique 22: 145–156.

———, AND S. YOUNG. 1980. Chronic wasting disease of captive mule deer: A spongiform encephalopathy. Journal of Wildlife Diseases 16: 89–98.

Received for publication 23 June 2006.