Polymorphisms of the prion protein gene (PRNP) in Alaskan moose (Alces alces gigas)

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Source/description: The prion protein (PRNP) gene of mammals encodes a prion protein (PrP), which is expressed in many tissues including the brain. Misfolded PrP conformers are responsible for neurodegenerative diseases known as spongiform encephalopathies. Transmissible spongiform encephalopathies (TSEs) include bovine spongiform encephalopathy, ovine scrapie, human Creutzfeldt–Jakob disease and chronic wasting disease (CWD) in mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus) and Rocky Mountain elk (Cervus elaphus). First found in Colorado, CWD has now been identified in the eastern USA, as far south as New Mexico and as far north as west-central Canada. Polymorphisms of PRNP appear to be linked to susceptibility to TSE in numerous species including free-ranging white-tailed deer and mule deer. In mule deer, the SS genotype at residue 225 is associated with a higher incidence of CWD. Differences in PrP amino acid sequence are believed to be species barriers to disease transmission. However, Wyoming moose sequences that were previously deposited in GenBank (AY225484 and AY225485) are similar to the sequence of Odocoileus. CWD has not been observed in Rocky Mountain moose (Alces alces shirasi) or in caribou at higher latitudes (Rangifer tarandus), yet both species overlap the geographical range of Odocoileus species. We report here the PRNP sequences for 44 Alaskan moose (Alces alces gigas).

Polymerase chain reaction conditions and sequence analysis: Genomic DNA was purified from blood samples of 44 moose (Alces alces gigas) that were sampled from eight locations across Alaska (Fig. S1). DNA purification protocols, primers, amplification conditions and sequence analysis methods are provided in Appendix S1.

Polymorphisms: Two unique sequences (i.e. alleles) were found in the sequences of 44 individual moose (DQ154297 and DQ154298); these differed only at codon 209. The allele encoding methionine was present with a frequency of 0.45, and the allele encoding isoleucine was present with a frequency of 0.55. The diploid genotypes did not depart significantly from Hardy–Weinberg predictions ($\chi^2 = 0.4$, $P < 0.011$).

Comments: The conservation of amino acid sequences in the PrP of moose, caribou and deer is striking (Table 1) and consistent with the fact that all three genera are in the subfamily

References

1. Rohrer G. A. et al. (1999) J Anim Sci 76, 1385–91.
2. Rathje T. A. et al. (1998) J Anim Sci 76, 1486–94.
3. Splan R. K. et al. (1998) J Anim Sci 76, 658–9.
4. Hiendlüer S. et al. (2002) Anim Genet 33, 247–8.
5. Harrison C. A. et al. (2005) Trends Endocrinol Metab 16, 73–8.
6. Knox R. V. et al. (2003) J Anim Sci 81, 249–60.
7. Christenson L. K. et al. (2000) Biochim Biophys Acta 1529, 175–87.
8. Pilon N. et al. (1997) Endocrinology 138, 1085–91.
9. LaVoie H. A. et al. (2004) Endocrinology 145, 3122–34.
10. Rohrer G. A. et al. (1996) Genome Res 6, 371–91.

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Supplementary Material

This following supplementary material is available as part of the online article from http://www.blackwell-synergy.com:
Table S1 PCR primers for STSs in porcine INHA and STAR.

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Capreolinae. In comparison with caribou, Alaskan moose samples show six synonymous substitutions (bases 195, 231, 324, 360, 384 and 674), presumably reflecting purifying selection for the unique conformation of the globular N-terminal domain of cervid prions. CWD has been transmitted to moose by an oral route in an experimental laboratory setting. Genetic similarities, susceptibility in the laboratory setting and overlapping geographical ranges suggest the lack of a barrier to the transmission of prion disease from mule and white-tailed deer to moose. The absence of reports of CWD transmission to moose in natural settings may reflect ecological or epidemiological factors. Moose tend to be more solitary than deer of the genus Odocoileus, and dense social aggregations might be prerequisites of CWD epizootic outbreaks in cervids.

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References
1 Prusiner S. B. (1998) Proc Natl Acad Sci U S A 95, 13363–83.
2 Weissmann C. (2004) Nat Rev Microbiol 2, 861–71.
3 Miller M. W. et al. (2000) J Wildlife Dis 36, 676–90.
4 Jewell J. E. et al. (2005) J Gen Virol 86, 2127–34.
5 Johnson C. et al. (2003) J Wildlife Dis 39, 576–81.
6 Raymond G. J. et al. (2000) EMBO J 19, 4425–30.
7 Gossert A. D. et al. (2005) Proc Natl Acad Sci U S A 102, 646–50.
8 Williams E. S. (2005) Vet Pathol 42, 530–49.

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Appendix S1 Table of differences among prion alleles, Materials and Methods.

Table 1 A comparison of differences among the prion protein alleles of moose (DQ154297 and DQ154298), caribou (DQ154293) and mule deer (AY228473).

| Nucleotide position | 4 | 195 | 231 | 324 | 360 | 384 | 385 | 413 | 438 | 505 | 618 | 627 | 674 |
|---------------------|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Moose               | G | A   | G   | C   | A   | T   | G   | G   | T   | G   | T   | G/T | T   |
| Caribou             | G/A| G   | T   | A   | C   | C   | G/A | G/A | C/T | G/A | T   | G   | C   |
| Mule deer           | A | A   | T   | A   | A   | C   | G   | G   | T   | G   | C   | G   | C   |

| Codon | 2 | 65 | 77 | 108 | 120 | 128 | 129 | 138 | 146 | 169 | 206 | 209 | 225 |
|-------|---|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Moose | V | G   | G   | P   | A   | L   | G   | S   | N   | V   | I   | M/I | S   |
| Caribou| V/M| G   | G   | P   | A   | L   | G/S | S/N | N   | V/M | I   | M   | S   |
| Mule deer| V | G   | G   | P   | A   | L   | G   | S   | N   | V   | I   | M   | S   |

Unlisted bases and amino acids were identical.

Figure S1 Locations of sites where moose were sampled in Alaska.

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Microsatellite CTSBJ12 is located distal to the ovine prion protein gene on OAR13 and is not associated with scrapie susceptibility

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Source/description: In order to determine the genetic localization of the ovine prion protein (PRNP) gene, 348 samples from one Merinoland and three Rhoen half-sib families (n = 114, 34, 23 and 20 lambs respectively) belonging to the breeding flock of the Research Station Oberer Hardthof of the Justus-Liebig University of Giessen were analysed for PRNP haplotypes (codons 136, 154 and 171) and microsatellites CTSBJ12, HUJ616 and URB58. To assess the association of CTSBJ12 alleles with susceptibility to scrapie, 137 pairs of scrapie-positive sheep and scrapie-negative or clinically healthy (in the following referred to as negative) flock mates belonging to 54 scrapie-affected German herds were analysed. The Bio-Rad Platelia rapid test (Bio-Rad Laboratories, Hercules, CA, USA), the Prionics Check Western Blot test (Prionics AG, Schlieren, Switzerland) and the Enfer TSE test (Enfer Scientific Limited, Newhall, Ireland) were used in 2002–2004 to identify scrapie-positive slaughtered or fallen stock and to confirm the negative scrapie status of culled flock mates. The final diagnosis of scrapie-positive sheep has been described elsewhere. Since 2003,