Gene Reports 4 (2016) 213–217

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Genetic variation of the prion protein gene (PRNP) in alpaca (*Vicugna pacos*)

M.S. Vermette [a], J.A. Schleining [b], J.J. Greenlee [c], J.D. Smith [a,*]

[a] Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, 1600 S 16th St., Ames, IA, USA
[b] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, 1600 S 16th St., Ames, IA, USA
[c] Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, 1920 Dayton Ave., Ames, IA, USA

ARTICLE INFO

Article history:
Received 30 December 2015
Received in revised form 5 June 2016
Accepted 7 June 2016
Available online 8 June 2016

Keywords:
Camelid
TSE
Spongiform
Encephalopathy

ABSTRACT

Transmissible spongiform encephalopathies (TSE) are caused by accumulation of a misfolded form of the prion protein (PrP). The normal cellular isoform of PrP is produced by the prion gene (*PRNP*) and is highly expressed in the central nervous system. Currently, there is an absence of information regarding the genetic sequence of alpaca *PRNP* and the potential susceptibility of this species to TSE. The objective of this study was to sequence the open reading frame of the alpaca prion gene and analyze this sequence for variation within the alpaca population and for homology to TSE-susceptible species. We sequenced the open reading frame of the prion gene of 40 alpacas of Huacaya or Suri descent. Length polymorphisms were identified within the sampled population. A subset (15%) of animals contained an additional 24 base pairs within the putative octapeptide repeat region. This polymorphism was independent of breed and sex. The majority (52.5%) of animals were heterozygous, possessing both longer and shorter alleles. Comparison with proven TSE-susceptible species (sheep, cattle, deer) revealed the following amino acid sequence variations: I6M, A16V, M17T, G92del, Q95_G96insG, N111S, R167K, N/T177S, I206V, S225Y, Y228S, Q230G, and L237del. Sequence alignment showed high homology compared to camel (>95%), sheep (>88%), cattle (>87%) and deer (>88%) *PRNP* sequence. This study demonstrates intraspecies variability within the *PRNP* open reading frame in alpacas and overall high sequence homology to TSE-susceptible species, providing foundational data for further research on the potential susceptibility of alpacas to TSE.

1. Introduction

Transmissible spongiform encephalopathies (TSE), also known as prion diseases, are a group of fatal neurodegenerative diseases that affect both animals and humans. These diseases include scrapie in sheep and goats, Creutzfeldt-Jakob disease in humans, chronic wasting disease (CWD) in deer and elk, and bovine spongiform encephalopathy (mad cow disease) in cattle. The prion protein (PrP^{C}), encoded by the prion gene (*PRNP*), is detergent-soluble and sensitive to proteinase K digestion. This protein is normally expressed in humans and animals, with greatest levels of expression in the central nervous system. It is generally recognized that prions, composed of PrP^{Sc} (the misfolded, disease-associated form of the prion protein), are formed from PrP^{C} by a posttranslational process that results in a profound change in conformation (Prusiner, 2013). The transformation and accumulation of PrP^{Sc}, a detergent insoluble form that is relatively resistant to proteases, is considered the central event in TSE pathogenesis (Aguzzi et al., 2001; Xu et al., 2012). Sequence analysis of *PRNP* in a number of animal species has demonstrated a high degree of conservation across mammals, typically with >50% sequence identity relative to humans (Schatzl et al., 1995; Wopfner et al., 1999).

Prion transmission within the same species is an efficient process, whereas transmission between species is usually less efficient or not possible ('species-barrier' phenomenon) (Hagiwara et al., 2013). However, the barrier sometimes can be overcome or negligible depending on the combination of donor and recipient animal PrP, or adaptation of prion strains to new hosts. For example, the CWD agent of mule deer has been experimentally transmitted by intracranial (IC) inoculation to cattle by first and second passage. On first passage, only 5 of 13 inoculated animals (38%) showed evidence of PrP^{Sc} amplification within 2 to 5 years post-inoculation (Hamir et al., 2005). Second passage resulted in 100% of inoculated cattle succumbing to infection within 16.5 months post-inoculation (Hamir et al., 2006a), demonstrating interspecies transmission and adaptation of the agent to the bovine host. Experimental interspecies transmission of the scrapie agent to cervids has also been achieved (Greenlee et al., 2011), demonstrating interspecies transmission potential for this TSE agent as well. These findings could be of concern to the alpaca industry given the potential

* Corresponding author at: Veterinary Pathology, 2714 Vet Med Bldg, 1600 S 16th St., Ames, IA 50011-1250, United States.
E-mail address: jdismith@iastate.edu (J.D. Smith).

http://dx.doi.org/10.1016/j.genrep.2016.06.004
2452-0144/

for physical interaction between alpacas and CWD-infected free-ranging cervids and/or scrapie-infected domestic sheep.

The alpaca is a part of the family Camelidae, along with the dromedary camel, Bactrian camel, llama, guanaco, and vicugna. There are two breeds of alpaca, Suri and Huacaya, both of which are found in the United States. These animals are used for fiber production and are considered the most color-diverse fiber-producing animals in the world. To date, there have been no reported cases of TSE in the alpaca. Whether this is a function of resistance of this species to TSE agents in general, underdiagnosis of alpacas with neurologic disease, or the relatively small US alpaca population and, therefore, exposure of a small number of animals to TSE agents, such as CWD and scrapie, remains to be determined. There is an absence of information about *PRNP* in the alpaca (*Vicugna pacos*). Within the Camelidae family, *PRNP* sequence has only been reported for Bactrian and dromedary camel (Kaluz et al., 1997; Xu et al., 2012). The aim of the current study was to elucidate the alpaca *PRNP* sequence and compare it to the *PRNP* sequences of other domesticated animals with proven susceptibility to TSE agents.

2. Materials and methods

Blood was collected from 40 alpacas ranging in age from 2 to 13 years and representing both Suri ($n = 20$) and Huacaya ($n = 20$) breed types (Table 1). Blood was obtained from the jugular vein via standard venipuncture procedure, placed into EDTA, and stored at 4 °C or −20 °C until processing. Genomic DNA was extracted from whole blood samples using the Roche HighPure PCR Template Preparation Kit (Roche Diagnostics Corporation, Indianapolis, IN, USA) and amplified by PCR using primer pairs (forward primer (7064F) 5′-TAGGACGC TGACACCCTCTT-3′ and reverse primer (R1) 5′-CCCACTATGAGGAAAA TGAG-3′; Integrated DNA Technologies, Coralville, IA) designed based on a combination of published camelid *PRNP* sequence data (Xu et al., 2012) and initial data analysis using an alternate forward primer (5′ ATCCTGGTTCTCTTTGTGGT-3′). Thermal cycling parameters comprised an initial denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 20 s, annealing at 58 °C for 20 s, and extension at 72 °C for 1 min, and ended by a final 10 min extension at 72 °C on an Applied Biosystems thermal cycler (Life Technologies, Carlsbad, CA) under standard buffer conditions (Herculase II Fusion DNA Polymerase, Agilent, Santa Clara, CA, USA). Each reaction contained 5 μL 5× Herculase II Reaction Buffer, 0.5 μL deoxyribonucleotide triphosphates (dNTPs), 0.63 μL of 7064F and R1 primers, 1.25 uL Dimethylsulfoxide (DMSO), 14.74 μL distilled water, 0.25 μL Herculase II fusion DNA polymerase, and 2 μL of extracted DNA. PCR products were confirmed by electrophoresis on 1% agarose gel and imaged using a gel imaging system (G:BOX; Syngene, Frederick, MD, USA) with GelRed DNA stain (GelRed, Phoenix Research, Candler, NC, USA). Samples resulting in more than one band on gel electrophoresis were separated using stab extraction with a 20 μL pipette tip. The sample was then pipetted into 100 μL of UltraPure DNase/RNase-free distilled water (Life Technologies, Carlsbad, CA, USA) and frozen at −20 °C until use. The samples were amplified using PCR parameters as described above. PCR products were then diluted at 1:10 with UltraPure DNase/RNase-free distilled water and analyzed using a 1% agarose gel to confirm a clean extraction. PCR products were then purified using an Amicon Ultra-0.5 mL centrifugal filter (EMD Millipore, Billerica, MA, USA) to remove unincorporated dNTPs and primers, then sequenced using Applied Biosystems 3100 genetic analyzer (Life Technologies, Carlsbad, CA, USA) with Big Dye Terminator chemistry (PE-Applied Biosystems, Carlsbad, CA, USA) using primers 7064F and R1. Initial sequence analysis revealed the necessity of additional primers to ensure accurate sequence identity; therefore, internal primers were created using the dromedary camel *PRNP* sequence (*Camelus dromedarius*, GenBank accession number Y09760) and Geneious version R6 (Biomatters, Auckland, New Zealand; Kearse et al., 2012). These primers were named based on location within the camel *PRNP* and included: 306F (5′-GAACAAGCCCAGTA AGCCGA-3′), 620F (5′-AGATGATGGAGCGCGTAGTG-3′), 327R (5′TTTC GGCTTACTGGGCTTGT-3′), 453R (5′-ACGGTCCTCATAGTCGTTGC-3′), 493R (5′-ACACTTGGTTGGGGTAACGG-3′), and 606R (5′-GGTGAAGTTC TCCCCCTTGG-3′). These primers provided overlapping sequence data (≥3× coverage) using the same sequence techniques described above. All sequence data was then analyzed and aligned against currently available GenBank consensus sequences of *PRNP* for cattle (*Bos taurus*, GenBank accession number FJ907304), sheep (*Ovis aries*, GenBank accession number HM803994), dromedary camel (*Camelus dromedarius*, GenBank accession number Y09760), Bactrian camel (*Camelus bactrianus*, GenBank accession number HQ204566.1), red deer (*Cervus elaphus*, GenBank accession number Y09761.1), and white-tailed deer (*Odocoileus virginianus*, GenBank accession number AF156185.1) to determine percent identity (pairwise alignment) using Geneious version R6.

Table 1
Animal information.

Bands by gel electrophoresis	Sample #	Age (yrs)	Sex	Breed
Double	11	6	F	Huacaya
	12	6	F	Huacaya
	14	2	F	Huacaya
	15	9	F	Huacaya
	16	6	F	Huacaya
	18	5	F	Huacaya
	22	6	F	Huacaya*
	24	7	F	Huacaya*
	26	10	F	Huacaya*
	29	5	F	Huacaya*
	30	5	F	Huacaya*
	23	13	M	Huacaya*
	27	7	M	Huacaya*
	32	Unk**	C/M	Suri
	36	Unk**	C/M	Suri
	37	Unk**	C/M	Suri
	39	Unk**	C/M	Suri
	1	8	F	Suri
	2	9	F	Suri
	6	7	F	Suri
	10	Unk**	F	Suri
High MW/long	13	5	F	Huacaya
	17	10	F	Huacaya
	21	7	M	Huacaya*
	25	11	M	Huacaya*
	31	Unk**	C/M	Suri
	33	Unk**	C/M	Suri
Low MW/short	19	5	F	Huacaya
	20	7	F	Huacaya
	28	7	M	Huacaya*
	34	Unk**	C/M	Suri
	35	Unk**	C/M	Suri
	38	Unk**	C/M	Suri
	40	Unk**	C/M	Suri
	3	11	F	Suri
	4	9	F	Suri
	5	10	F	Suri
	7	8	F	Suri
	8	7	F	Suri
	9	6	F	Suri

Unk = unknown, F = female, M = male, C/M = castrated male.
* Accoyo line.
** ≥6 years of age.

3. Results

Complete alpaca open reading frame *PRNP* nucleotide sequence and deduced PrP amino acid sequence were determined (Fig. 1). Sequence data was submitted to NCBI's GenBank (accession numbers KT692714 and KT692715). On gel electrophoresis, variability was discovered among PCR products (Fig. 2). Three distinct banding patterns were observed: DNA fragments from 6 alpacas (15%) had a higher molecular weight (approximately 800 bp), DNA fragments from 13 alpacas

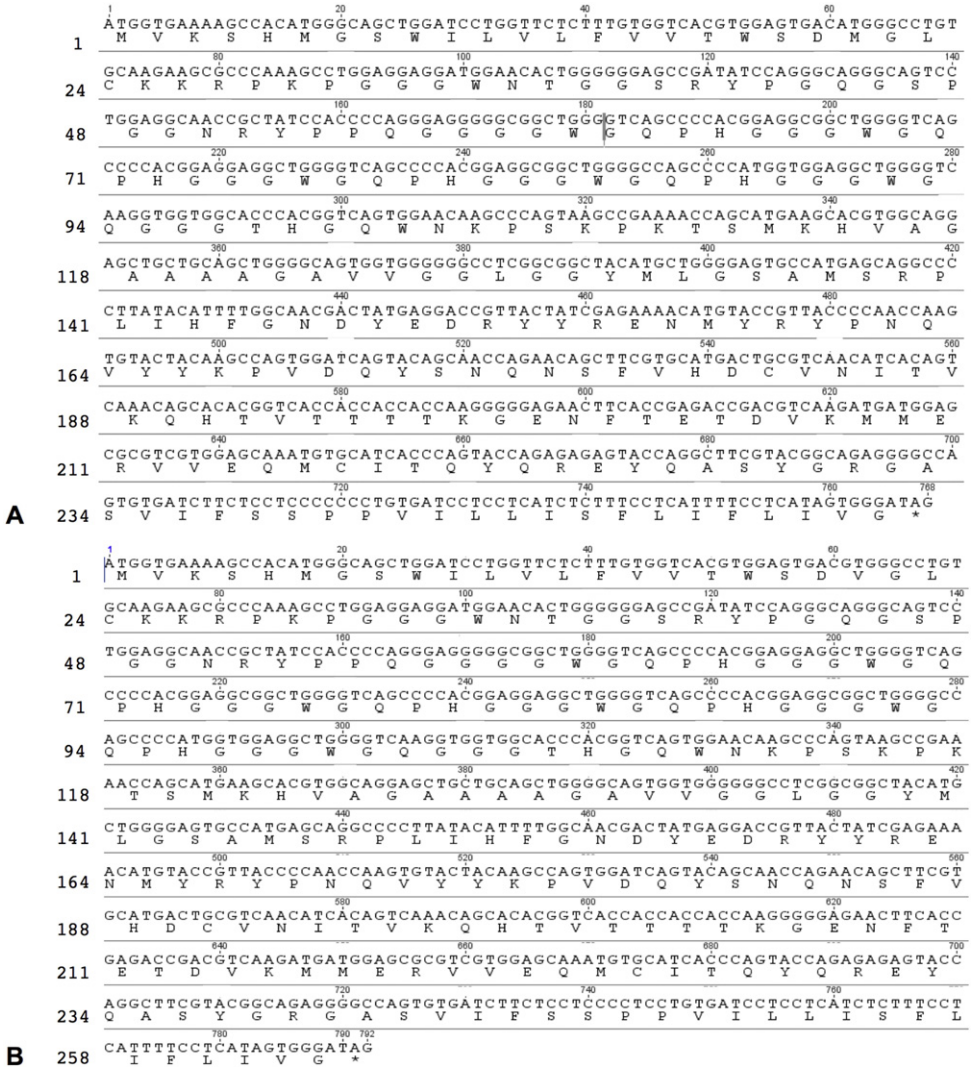

Fig. 1. Nucleotide and deduced amino acid sequences of the alpaca *PRNP* open reading frame. A. Low molecular weight sample sequence (GenBank accession KT692714). B. High molecular weight sample sequence (GenBank accession KT692715).

(32.5%) had a lower molecular weight (approximately 750 bp), and DNA fragments from 21 alpacas (52.5%) separated into two distinct bands, which were of similar size to the single high and low band samples. These length variations were independent of both breed and sex (Table 1). Sequence analysis of a subset of high (samples 13, 17, 25) and low (samples 3, 4, 5) molecular weight samples revealed the presence of an additional 24 base pairs (CCCCACGGAGGCGGCTGGGGTCAG; deduced amino acid sequence PHGGGWGQ) in the high molecular weight samples within the octa(nona)peptide repeat region, and a single difference at amino acid residue 21 (M21V) (Fig. 1). Alpacas with the shorter allele had an octa(nona)peptide repeat region composed of an initial nonapeptide followed by four octapeptide repeats: PQGGGGWGQ- PHGGGWGQ- PHGGGWGQ- PHGGGWGQ- PHGGGWGQ. Alpacas with the longer allele had a similar octa(nona)peptide repeat region organization but with one additional octapeptide repeat. PCR products yielding double bands on the agarose gel were separated by stab extraction and each band was analyzed individually. Nucleotide sequence for each band was highly homologous (99.9%) to that identified

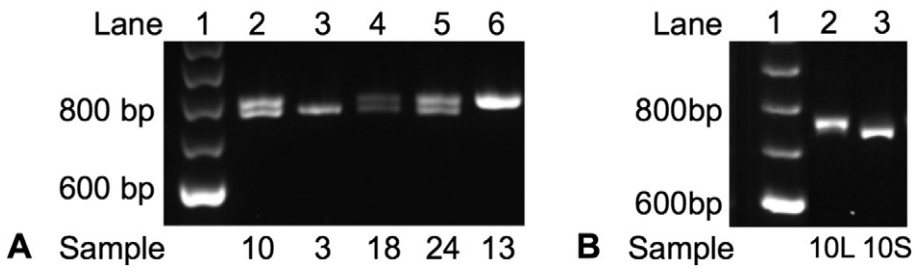

Fig. 2. Gel electrophoresis of PCR products on 1% agarose gel. A) Representative images of PCR product length polymorphisms. Lane 3: PCR product from an alpaca homozygous for the lower molecular weight/shorter allele. Lane 6: PCR product from an alpaca homozygous for the higher molecular weight/longer allele. Lanes 2, 4, and 5: PCR products exhibiting two distinct bands (heterozygotes). Lane 1: DNA ladder. B) PCR products from Lane 2 (sample #10) in (A) after stab extraction. PCR amplification of the extracted products yielded two distinct bands.

for the single high and low molecular weight DNA fragments, with the higher molecular weight band similarly containing one additional PHGGGWGQ repeat.

Alpaca *PRNP* and deduced PrP sequence was compared to other camelids and a variety of TSE-susceptible species. Sequence of the shorter allele (4 octapeptide-containing) was highly homologous to both dromedary and Bactrian camel with nucleotide and amino acid homologies of 98.4% and 99.2% to both camelid species. Alpaca *PRNP* and PrP sequences of the shorter allele were also closely related to sheep (89.9 and 93.4%), red deer (90.4 and 93.4%), white-tailed deer (90.1 and 93.8%), and cattle (longer bovine allele; 87.3 and 90.9%). Nucleotide and amino acid homologies for the longer allele (5 octapeptide-containing) were similarly related to camel (96.1 and 96.6% Bactrian; 96.0 and 96.6% dromedary), sheep (88.2 and 91.7%), red deer (88.7 and 91.7%), white-tailed deer (88.3 and 92.0%), and cattle (longer bovine allele; 90.0 and 94.0%). Several amino acid changes were identified in alpaca PrP sequence compared to other non-camelid species: I6M, A16V, M17T, G92del, Q95_G96insG, N111S, R167K, N/T177S, I206V, S225Y, Y228S, Q230G, and L237del (sheep notation) (Fig. 3).

4. Discussion

In the current study, we report the nucleotide sequence of *PRNP* and deduced primary structure of PrP in the alpaca. Our results demonstrate variation within the alpaca population in the form of length polymorphisms, with 15% of the sampled population containing an additional 24 base pairs in the putative octa(nona)peptide repeat region, and *PRNP* heterozygosity, with greater than 50% possessing both the longer and shorter alleles. Alpacas with the shorter allele, similar to camels, had an octa(nona)peptide repeat region composed of an initial nonapeptide followed by four octapeptide repeats. Alpacas with the longer allele had a similar octa(nona)peptide repeat region organization but with five octapeptide repeats following the initial nonapeptide. In addition to alpacas, longer alleles with six instead of five repeats have also been identified in cattle (Goldmann et al., 1991). Goldmann et al. also identified heterozygous cattle with a 6 and 5 octa(nona)peptide genotype similar to that observed here in alpacas. Susceptibility to TSE can be influenced by the number of *PRNP* octapeptide repeats. Publications investigating insertional mutations in the octa(nona)peptide region of human *PRNP* suggest that there is a higher likelihood of prion protein aggregation in patients with additional octapeptide repeats (Yu et al., 2007). Another study in transgenic mice expressing bovine *PRNP* showed that four extra octapeptide repeats accelerated disease with the bovine spongiform encephalopathy agent (Castilla et al., 2005). No difference has been reported in the frequencies of five or six copies of the octapeptide repeat sequence in healthy cattle and cattle with BSE (Hunter et al., 1994); however, Brown Swiss cattle can encode 7 octapeptide repeats (Schlapfer et al., 1999; Seabury et al., 2004), which has been associated with an increased susceptibility to BSE (Geldermann et al., 2006; Sauter-Louis et al., 2006). The significance of an additional octapeptide repeat in a subset of the alpaca population is unknown. Any future studies examining the potential susceptibility of this species to TSE agents would need to take this variation into account.

Sequence analysis revealed that alpaca *PRNP* has high sequence homology when compared to either dromedary or Bactrian camel (>96%), and moderately high sequence homology to TSE-susceptible species (>87%), but with a number of amino acid differences. Genetic determinants of prion susceptibility and resistance have been identified in a number of species including sheep (Hunter et al., 1996), goats (Goldmann et al., 1996), elk (Hamir et al., 2006b), and humans (Palmer et al., 1991). For example, scrapie commonly affects sheep homozygous for Q^{171} versus R^{171} (Baylis et al., 2002). Polymorphisms present in the alpaca, as well as other camelids examined to date, compared to TSE-susceptible species may contribute to TSE resistance as no

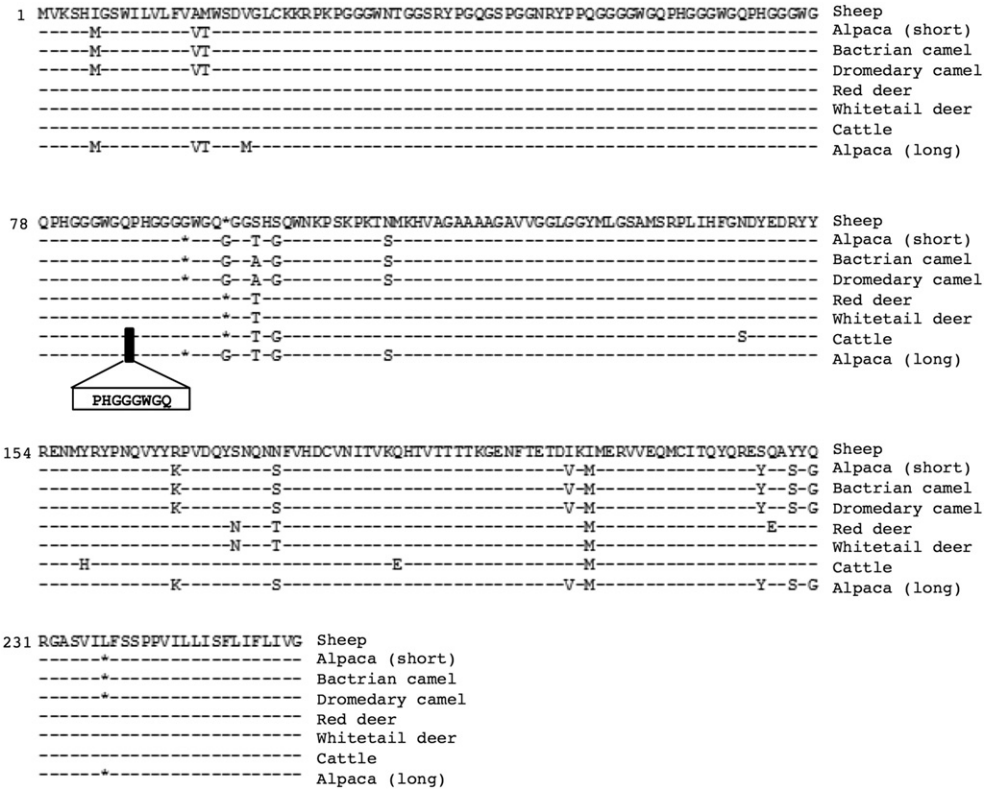

Fig. 3. Alignment of PrP sequences of 8 mammalian prion proteins. 1. Sheep (*Ovis aries*, HM803994), 2. Alpaca low molecular weight sample/short allele, 3. Bactrian camel (*Camelus bactrianus*,HQ204566.1), 4. Dromedary camel (*Camelus dromedarius*, Y09760), 5. Red deer (*Cervus elaphus*, Y09761.1), 6. White-tailed deer (*Odocoileus virginianus*, AF156185.1), 7. Cattle (*Bos taurus*, FJ907304), 8. Alpaca high molecular weight sample/long allele. Sites identical to the consensus sequence (sheep) are denoted by dashes (—), and sites with a deletion by asterisks (*).

known cases of prion disease have been documented in camelids. Further studies investigating the susceptibility of alpacas to TSE agents are required to address this question.

Abbreviations

A	adenosine
bp	base pair(s)
C	cytidine
CWD	chronic wasting disease
DMSO	dimethylsulfoxide
DNase	deoxyribonuclease
dNTP	deoxyribonucleoside triphosphate
G	guanosine
IC	intracranial
PCR	polymerase chain reaction
PRNP	prion protein gene
PrP/PrP^{C}	prion protein
PrP^{Sc}	misfolded prion protein
RNase	ribonuclease
T	thymidine
TSE	transmissible spongiform encephalopathy

Acknowledgments

The authors thank Kevin Hassall and Dr. David Alt for excellent technical assistance. This work was partially supported by the Iowa State University College of Veterinary Medicine Van Gelder Fund to support student research. Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture. USDA is an equal opportunity provider and employer.

References

Aguzzi, A., Glatzel, M., Montrasio, F., Prinz, M., Heppner, F.L., 2001. Interventional strategies against prion diseases. Nat. Rev. Neurosci. 2, 745–749.

Baylis, M., Goldmann, W., Houston, F., Cairns, D., Chong, A., Ross, A., Smith, A., Hunter, N., McLean, A.R., 2002. Scrapie epidemic in a fully PrP-genotyped sheep flock. J. Gen. Virol. 83, 2907–2914.

Castilla, J., Gutierrez-Adan, A., Brun, A., Pintado, B., Salguero, F.J., Parra, B., Segundo, F.D., Ramirez, M.A., Rabano, A., Cano, M.J., Torres, J.M., 2005. Transgenic mice expressing bovine PrP with a four extra repeat octapeptide insert mutation show a spontaneous, non-transmissible, neurodegenerative disease and an expedited course of BSE infection. FEBS Lett. 579, 6237–6246.

Geldermann, H., He, H., Bobal, P., Bartenschlager, H., Pruess, S., 2006. Comparison of DNA variants in the PRNP and NF1 regions between bovine spongiform encephalopathy and control cattle. Anim. Genet. 37, 469–474.

Goldmann, W., Hunter, N., Martin, T., Dawson, M., Hope, J., 1991. Different forms of the bovine PrP gene have five or six copies of a short, G-C-rich element within the protein-coding exon. J. Gen. Virol. 72, 201–204.

Goldmann, W., Martin, T., Foster, J., Hughes, S., Smith, G., Hughes, K., Dawson, M., Hunter, N., 1996. Novel polymorphisms in the caprine PrP gene: a codon 142 mutation associated with scarpie incubation period. J. Gen. Virol. 77, 2885–2891 (erratum 1997, 78, 679).

Greenlee, J.J., Smith, J.D., Kunkle, R.A., 2011. White-tailed deer are susceptible to the agent of sheep scrapie by intracerebral inoculation. Vet. Res. 42, 107.

Hagiwara, K., Hara, H., Hanada, K., 2013. Species-barrier phenomenon in prion transmissibility from a viewpoint of protein science. J. Biochem. 153, 139–145.

Hamir, A.N., Kunkle, R.A., Cutlip, R.C., Miller, J.M., O'Rourke, K.I., Williams, E.S., Miller, M.W., Stack, M.J., Chaplin, M.J., Richt, J.A., 2005. Experimental transmission of chronic wasting disease agent from mule deer to cattle by the intracerebral route. J. Vet. Diagn. Investig. 17, 276–281.

Hamir, A.N., Kunkle, R.A., Miller, J.M., Greenlee, J.J., Richt, J.A., 2006a. Experimental second passage of chronic wasting disease (CWD(mule deer)) agent to cattle. J. Comp. Pathol. 134, 63–69.

Hamir, A.N., Gidlewski, T., Spraker, T.R., Miller, J.M., Creekmore, L., Crocheck, M., Cline, T., O'Rourke, K.I., 2006b. Preliminary observations of genetic susceptibility of elk (*Cervus elaphus nelsoni*) to chronic wasting disease by experimental oral inoculation. J. Vet. Diagn. Investig. 18, 110–114.

Hunter, N., Goldmann, W., Smith, G., Hope, J., 1994. Frequencies of PrP gene variants in healthy cattle and cattle with BSE in Scotland. Vet. Rec. 135, 400–403.

Hunter, N., Foster, J.D., Goldmann, W., Stear, M.J., Hope, J., Bostock, C., 1996. Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PRP genotypes. Arch. Virol. 141, 809–824.

Kaluz, S., Kaluzova, M., Flint, A.P., 1997. Sequencing analysis of prion genes from red deer and camel. Gene 199, 283–286.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.

Palmer, M.S., Dryden, A.J., Hughes, J.T., Collinge, J., 1991. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. Nature 353, 340–342.

Prusiner, S.B., 2013. Biology and genetics of prions causing neurodegeneration. Annu. Rev. Genet. 47, 601–623.

Sauter-Louis, C., Clauss, M., Chaher, E., Klee, W., Wichmann, H.E., Kienzle, E., 2006. Breed predisposition for BSE: epidemiological evidence in Bavarian cattle. Schweiz. Arch. Tierheilkd. 148, 245–250.

Schatzl, H.M., Da Costa, M., Taylor, L., Cohen, F.E., Prusiner, S.B., 1995. Prion protein gene variation among primates. J. Mol. Biol. 245, 362–374.

Schlapfer, I., Saitbekova, N., Gaillard, C., Dolf, G., 1999. A new allelic variant in the bovine prion protein gene (PRNP) coding region. Anim. Genet. 30, 386–387.

Seabury, C.M., Honeycutt, R.L., Rooney, A.P., Halbert, N.D., Derr, J.N., 2004. Prion protein gene (PRNP) variants and evidence for strong purifying selection in functionally important regions of bovine exon 3. Proc. Natl. Acad. Sci. U. S. A. 101, 15142–15147.

Wopfner, F., Weidenhofer, G., Schneider, R., von Brunn, A., Gilch, S., Schwarz, T.F., Werner, T., Schatzl, H.M., 1999. Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein. J. Mol. Biol. 289, 1163–1178.

Xu, L., Zhang, Z., Wang, L., Feng, D., Zhou, X., Xu, B., Zhao, D., 2012. Cloning and polymorphism analysis of prion protein gene in domestic bactrian camel in China. Gene 491, 256–259.

Yu, S., Yin, S., Li, C., Wong, P., Chang, B., Xiao, F., Kang, S.C., Yan, H., Xiao, G., Tien, P., Sy, M.S., 2007. Aggregation of prion protein with insertion mutations is proportional to the number of inserts. Biochem. J. 403, 343–351.