VETERINARIA ITALIANA
RIVISTA DI SANITÀ PUBBLICA VETERINARIA

Paper

Genetic characterization of the Prion Protein Gene in the indigenous Nguni cattle breed of the Kingdom of Eswatini

Pumla Bhekiwe Manyatsi[1], Sedef Erkunt Alak[1], Hüseyin Can[1], Ahmet Efe Köseoğlu[1], Mervenur Güvendi[1], Cemal Ün[1*]

[1]*Ege University Faculty of Science Department of Biology Molecular Biology Section, İzmir - TR*

[*]*Corresponding author at: Ege University Faculty of Science Department of Biology Molecular Biology Section, İzmir - TR*
E-mail: cemaluen@gmail.com

Veterinaria Italiana, Vol. 61 No. 2 (2025) DOI: 10.12834/VetIt.3560.26921.2

Abstract

Bovine spongiform encephalopathy (BSE) is one of several fatal neurodegenerative diseases caused by misfolded, infectious prion proteins. Polymorphisms in the prion protein gene (PRNP) associated with BSE susceptibility have been reported in various cattle breeds. However, no studies have been conducted to investigate these genetic variations in the Nguni cattle breed, an indigenous breed in the Kingdom of Eswatini, Southern Africa. The present study aimed to genetically characterize the PRNP gene in Nguni cattle. Both the coding region and regulatory elements—specifically the promoter and intron 1 regions—were analyzed. Three synonymous polymorphisms were identified: Q78Q, P113P, and I226I. Additionally, the Nguni breed exhibited a higher frequency of deletion alleles in two known insertion/deletion (indel) polymorphisms: a 23-bp deletion in the promoter region and a 12-bp deletion in intron 1, compared to insertion alleles. These findings suggest that Nguni cattle may be genetically susceptible to BSE. The results underscore the importance of implementing selective breeding strategies aimed at enhancing resistance to BSE within this indigenous cattle population.

Keywords

BSE, Eswatini cattle, Indel, Prion, PRNP

Introduction

Bovine spongiform encephalopathy (BSE), commonly known as mad cow disease, is one of several fatal neurodegenerative disorders classified as transmissible spongiform encephalopathies (TSEs). In addition to BSE, TSEs include feline spongiform encephalopathy (FSE) in cats, scrapie in sheep, and Creutzfeldt-Jakob disease (CJD) and Kuru in humans (Prusiner, 1998; Prusiner, 2004). The causative agent of TSEs is the infectious prion protein (PrP^{Sc}), which is a misfolded isoform of the cellular prion protein (PrP^{C}) (Belay, 1999).

To date, numerous studies have investigated the relationship between prion gene polymorphisms and susceptibility to prion diseases. In sheep, polymorphisms at codons 136, 154, and 171 are associated with susceptibility to scrapie, while in humans, a polymorphism at codon 129 significantly influences the occurrence of variant CJD (Peden et al., 2004; Vaccari et al., 2001). Investigations into the relationship between BSE and the prion protein gene have revealed no polymorphisms within the PRNP coding region that are associated with susceptibility or resistance to typical BSE in cattle. However, the E211K polymorphism in the PRNP coding region—homologous to the E200K variant that causes CJD in humans—has been linked to susceptibility to atypical BSE (Nicholson et al., 2008; Heaton et al., 2008). Additionally, insertion/deletion (indel) polymorphisms in the promoter (23-bp) and intron 1 (12-bp) regions of the bovine PRNP gene have been associated with susceptibility to classical BSE. The deletion genotype has been reported in association with BSE susceptibility, whereas the insertion allele appears to be more prevalent in healthy animals (Murdoch and Murdoch, 2015; Murdoch et al., 2010).

Given that prion diseases are among the most serious and fatal conditions affecting both humans and livestock,

How to cite: Manyatsi et al. Genetic characterization of the Prion Protein Gene in the indigenous Nguni cattle breed of the Kingdom of Eswatini. Veterinaria Italiana, Vol. 61 No. 2 (2025) DOI: 10.12834/VetIt.3560.26921.2

investigating the frequency of known resistance- or susceptibility-associated alleles in at-risk populations is critical for understanding potential outbreak scenarios (Choi et al., 2012). In this context, the present study aimed to analyze the PRNP gene of Nguni cattle to assess genetic susceptibility or resistance to prion diseases. Nguni cattle are a commonly bred indigenous breed in the Kingdom of Eswatini, Southern Africa (Madilinni et al., 2020). To date, no cases of BSE have been reported in this breed. Moreover, the genetic characterization of the PRNP gene in Nguni cattle has not previously been investigated.

Therefore, we examined the PRNP coding region as well as the 12-bp and 23-bp indel polymorphisms in 25 Nguni cattle. In addition, we analyzed the allele and genotype frequencies and compared them with those observed in other cattle breeds.

Materials and methods

Ethics and Animals

All experiments were conducted in accordance with the ethical guidelines and approval of the Eswatini Ministry of Agriculture and Veterinary Headquarters (approval number: 2020/02/07/011MH). All methods employed in the study were carried out in compliance with relevant institutional and national regulations. Blood samples were collected from 25 randomly selected Nguni cattle using EDTA-containing tubes.

DNA isolation and PCR

Genomic DNA was isolated using PureLink® Genomic DNA Mini Kit according to the manufacturer's instructions. PCR amplification was carried out as described by Ün et al., (2008) using primers in Table I. During PCR, 25 µL reaction volume included 12.5 µl Dream Taq master mix (Thermo scientific), 2,5 µl genomic DNA sample, 1 µl from each primer (10 µM) and 8 µl molecular grade water and. The PCR targeting the coding region of PRNP gene was performed under the following conditions: 3 min initial denaturation step at 94 °C, followed by 35 cycles of 50 s at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, and a final extension of 10 min at 72 °C whereas the PCR targeting the promotor and intron 1 region of the PRNP gene was carried out under the following conditions: 5 min initial denaturation step at 95 °C, followed by 32 cycles of 45 s min at 94 °C, 45 s at 58 °C, and 45 s at 72 °C, and a final extension of 7 min at 72 °C. PCR products were run on 1 % agarose gel electrophoresis and visualised.

PCR Region	Forward Primer	Reverse Primer
Coding region of PRNP gene	5- AAAGCCACATAGGCAGTTG-3	5'- AATGAGGAAAGAGATGAGGAG-3'
The promotor (23-bp indel)	5'-CCTGTTGAGCGTGCTCGT-3'	5'-GGTAGAGGAGCCGCAGGT-3'
Intron 1 (12-bp indel)	5'-TGCCTGTCCAACTCTTTG-3'	5'-TGCCTGTCCAACTCTTTG-3'

Table I. Primers used in PCR reactions.

Genotyping

PCR products corresponding to the coding region of the bovine PRNP gene were sequenced using an ABI 3730XL DNA Analyzer. The resulting sequences were aligned using MEGA X software (Kumar et al., 2018) and compared with reference sequences (Accession numbers: DQ875184 and FJ907266) to identify polymorphisms. Additionally, the genotypes of the 23-bp and 12-bp indels were analyzed by electrophoresis on a 1% agarose gel, based on the size of the PCR products.

How to cite: Manyatsi et al. Genetic characterization of the Prion Protein Gene in the indigenous Nguni cattle breed of the Kingdom of Eswatini. Veterinaria Italiana, Vol. 61 No. 2 (2025) DOI: 10.12834/VetIt.3560.26921.2

Statistical analysis

Allele and genotype frequencies, Hardy-Weinberg equilibrium (HWE), and Chi-square tests were calculated using POPGENE32 software (Yeh, 2000).

Results

In the coding region of the PRNP gene, polymorphisms were identified at three positions: G234A, C339T, and T678C, when compared with the reference sequences. However, none of these polymorphisms resulted in amino acid substitutions in the corresponding protein sequences (Q78Q, P113P, and I226I). The allele frequencies for G234A, C339T, and T678C were 0.90, 0.92, and 0.92, respectively (Table II). Regarding SNP positions 234/339/678, the CAG/CCC/ATT genotype was predominant, observed in 16 samples, while the CAG/CCT/ATC and CAA/CCC/ATT genotypes were detected in 4 and 5 samples, respectively (Table III).

For the indel polymorphisms, the deletion allele was found at a higher frequency than the insertion allele at both loci in the study population. The deletion allele frequencies were 0.92 for the 12-bp indel and 0.76 for the 23-bp indel. For the 23-bp indel polymorphism, the insertion/insertion (II) genotype was the least frequent (n = 3), whereas the deletion/deletion (DD) genotype was the most common (n = 16). In the case of the 12-bp indel, the DD genotype was predominant, observed in 21 samples, and the II genotype was not detected (Table IV).

Polymorphism Positions	Alleles	Amino Acid Changes	Number of Samples	Allele Frequency
234	CA**G***	Q78Q	20	0.90
	CA**A**		5	0.10
339	CC**C***	P113P	21	0.92
	CC**T**		4	0.08
678	AT**T***	I226I	21	0.92
	AT**C**		4	0.08

Bold shows nucleotide conversion

*Alleles in the reference sequence

Table II. Allele frequencies of nucleotide polymorphisms identified in the coding region of the PRNP gene.

Breeds	SNP positions	Genotypes	Obs. (O)	Exp. (E)	$(O-E)^2/E$	$\chi 2$	*p*
Nguni	234/339/678	CAG/CCC/ATT	16	128.571	0.7683	9.928.205	0.001628
		CAG/CCT/ATC	4	102.857	38.413		
		CAA/CCC/ATT	5	18.571	53.187		

Table III. Hardy-Weinberg equilibrium analysis of PRNP single nucleotide polymorphisms in the Nguni cattle breed.

Breeds	Locus	Genotypes	N	Obs. (O)	Exp. (E)	$(O-E)^2/E$	$\chi 2$	p
Nguni	23bp indel	II	3	30.000	13.469	20.285	3.393.767	0.0818
		ID	6	60.000	93.061	11.745		
		DD	16	16.000	143.469	0.1905		
	12bp indel	II	0	0.0000	0.1224	0.1224	0.139130	0.7091
		ID	4	40.000	37.551	0.0160		
		DD	21	21.000	211.224	0.0007		

Table IV.Hardy-Weinberg equilibrium analysis of PRNP indel polymorphisms in the Eswatini native cattle breed. Genotypes: II = insertion/insertion, ID = insertion/deletion, DD = deletion/deletion.

How to cite: Manyatsi et al. Genetic characterization of the Prion Protein Gene in the indigenous Nguni cattle breed of the Kingdom of Eswatini. Veterinaria Italiana, Vol. 61 No. 2 (2025) DOI: 10.12834/VetIt.3560.26921.2

Discussion

To date, several studies have examined the association between the *PRNP* gene and BSE susceptibility in cattle (Sander et al., 2005). Most of these investigations have focused on identifying polymorphisms in both coding and non-coding regions of *PRNP* that influence resistance or susceptibility to BSE. Although the *PRNP* gene has been genetically characterized in most cattle breeds (Yaman and Ün, 2017), no studies have previously been conducted on the Nguni breed. Therefore, in this study, the *PRNP* gene of Nguni cattle was analyzed, and three synonymous polymorphisms—Q78Q (G234A), P113P (C339T), and T226T (T678C)—were identified. In addition, a high frequency of the deletion genotype for both the 23-bp and 12-bp indel polymorphisms, which have been associated with susceptibility to BSE, was observed.

The G234A polymorphism identified in our study has been reported as prevalent in both *Bos taurus* and *Bos indicus* breeds (Brunelle et al., 2008), and was also found in a previous study on Korean Hanwoo cattle (Jeong et al., 2006). Similarly, the P113P (C339T) and T226T (T678C) polymorphisms have been reported in Slovak and Chinese cattle, respectively (Hreško and Tkáčiková, 2011; Zhao et al., 2009). The E211K amino acid substitution, associated with atypical BSE susceptibility, was not detected in any of the Nguni samples analyzed.

Source Country	Breed	Allele Frequency				References
		23bp		12bp		
		I	D	I	D	
Eswatini	Nguni	0.24	0.76	0.08	0.92	Present study
Ethiopia	Borona	0.12	0.88	0.36	0.64	Teferedegn et al., 2022
	Fogera	0.10	0.90	0.45	0.55	
	Arsi	0.12	0.88	0.28	0.72	
	Afar	0.16	0.84	0.14	0.86	
China	Xia'nan	0.42	0.58	0.56	0.44	(Yang et al. 2018)
	Qinchuan	0.57	0.43	0.60	0.40	
	Nanyang	0.73	0.27	0.80	0.26	
	Jiaxian	0.61	0.40	0.73	0.27	
	Ji'an	0.30	0.70	0.67	0.33	
	Pi'nan	0.46	0.54	0.62	0.38	
Japan	Japanese Black Cattle	0.23	0.77	0.42	0.58	(Msalya et al.2011)
	Japanese Brown Cattle	0.44	0.56	0.47	0.53	
Iran	Iranian Holstein	0.37	0.63	0.52	0.48	(Maghsoodi et al. 2011)
	Golpayegani	0.30	0.70	0.95	0.05	
	Sistani	0.18	0.82	0.87	0.13	
Turkey	South Anatolian Red	0.36	0.64	0.69	0.31	(Ün, et al. 2008)
	East Anatolian Red	0.40	0.60	0.72	0.28	
	Gray	0.62	0.38	0.80	0.20	
German	BSE affected	0.27	0.73	0.33	0.67	(Haase et al. 2007)
	Healthy	0.43	0.57	0.49	0.51	
UK	UK Holstein case	0.25	0.75	0.28	0.72	(Juling et al. 2006)
	UK Holstein control	0.30	0.70	0.37	0.63	
Korean	Healthy/Holstein	0.30	0.70	0.39	0.62	(Jeong et al. 2006)

Table V. Allele frequencies and statistical comparisons for the 23-bp and 12-bp indels.

When comparing the indel allele frequencies observed in our study to those reported in other cattle breeds (Table V), the deletion allele was found at a relatively high frequency at both loci in the Nguni cattle population. In contrast,

How to cite: Manyatsi et al. Genetic characterization of the Prion Protein Gene in the indigenous Nguni cattle breed of the Kingdom of Eswatini. Veterinaria Italiana, Vol. 61 No. 2 (2025) DOI: 10.12834/VetIt.3560.26921.2

studies involving cattle from Turkey and Iran reported higher insertion allele frequencies for the 12-bp indel polymorphism, while the 23-bp deletion allele frequency was approximately 0.7 across those breeds (Ün et al., 2008; Maghsoodi et al., 2011). In Germany, the deletion allele was observed more frequently in BSE-affected animals than in healthy controls (Haase et al., 2007). Similarly, in Ethiopian cattle breeds, the deletion allele frequency was high at both loci, consistent with our findings (Teferedegn et al., 2022). These results may suggest that, in African cattle populations where no fatal prion disease outbreaks have been recorded, there has been no selective pressure for the enrichment of resistant genotypes.

The strength of this study lies in its being the first comprehensive characterization of the *PRNP* gene in Nguni cattle, an indigenous breed of Eswatini, despite the overall scarcity of data on prion disease resistance in African countries. However, a limitation of this study is the relatively small sample size, indicating the need for broader sampling in future research.

Conclusion

To the best of our knowledge, this is the first comprehensive investigation of the *PRNP* gene in Nguni cattle, an indigenous breed common in the Kingdom of Eswatini. The observation of a high frequency of the deletion allele at both loci (12-bp and 23-bp indels) is particularly noteworthy. These findings suggest that the Nguni breed may be genetically susceptible to BSE. This susceptibility could be attributed to evolutionary forces influencing *PRNP* gene variation or to the absence of previous exposure to fatal BSE outbreaks in this population.

Moreover, the results indicate that in the event of a prion disease outbreak, cattle losses could be substantial. Therefore, the development of resistant breeding lines is critical to mitigating such risks. In conclusion, further studies involving larger sample sizes across different regions of Eswatini are essential. These efforts will support the establishment of a BSE-resistant breeding strategy, including the registration and genetic monitoring of all herd members to inform sustainable breeding policies.

Funding Data

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contributions

Design of the study: CU. Carrying out the experiments: PBM, SEA, HC, AEK and MG. Data analysis: CU, PBM and SEA. Writing the article: PBM, SEA, HC and CU. Review and editing the article: CU, HC and SEA. All authors have read and approved the manuscript.

Competing interest statement

The authors declare that there are no potential conflicts of interest.

References

Belay E. D. (1999). Transmissible spongiform encephalopathies in humans. Annual review of microbiology, 53, 283–314. https://doi.org/10.1146/annurev.micro.53.1.283.

Brunelle, B. W., Greenlee, J. J., Seabury, C. M., Brown, C. E., 2nd, & Nicholson, E. M. (2008). Frequencies of polymorphisms associated with BSE resistance differ significantly between Bos taurus, Bos indicus, and composite cattle. BMC veterinary research, 4, 36. https://doi.org/10.1186/1746-6148-4-36.

Choi, S., Woo, H. J., & Lee, J. (2012). Sequence variations of the bovine prion protein gene (PRNP) in native Korean Hanwoo cattle. Journal of veterinary science, 13(2), 127–137. https://doi.org/10.4142/jvs.2012.13.2.127.

Haase, B., Doherr, M. G., Seuberlich, T., Drögemüller, C., Dolf, G., Nicken, P., Schiebel, K., Ziegler, U., Groschup, M. H., Zurbriggen, A., & Leeb, T. (2007). PRNP promoter polymorphisms are associated with BSE susceptibility in Swiss and German cattle. BMC genetics, 8, 15. https://doi.org/10.1186/1471-2156-8-15.

Heaton, M. P., Keele, J. W., Harhay, G. P., Richt, J. A., Koohmaraie, M., Wheeler, T. L., Shackelford, S. D., Casas, E., King, D. A., Sonstegard, T. S., Van Tassell, C. P., Neibergs, H. L., Chase, C. C., Jr, Kalbfleisch, T. S., Smith, T. P., Clawson, M. L., & Laegreid, W. W. (2008). Prevalence of the prion protein gene E211K variant in U.S. cattle. BMC veterinary research, 4, 25. https://doi.org/10.1186/1746-6148-4-25.

Hreško, S., & Tkáčiková, L. (2012). Variation in the coding region of the prion protein gene in Slovak cattle. Acta veterinaria Hungarica, 60(2), 233–243. https://doi.org/10.1556/AVet.2012.020.

Jeong, B. H., Lee, Y. J., Kim, N. H., Carp, R. I., & Kim, Y. S. (2006). Genotype distribution of the prion protein gene (PRNP) promoter polymorphisms in Korean cattle. Genome, 49(12), 1539–1544. https://doi.org/10.1139/g06-110.

Juling, K., Schwarzenbacher, H., Williams, J. L., & Fries, R. (2006). A major genetic component of BSE susceptibility. BMC biology, 4, 33. https://doi.org/10.1186/1741-7007-4-33.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular biology and evolution, 35(6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Nicholson, E. M., Brunelle, B. W., Richt, J. A., Kehrli, M. E., Jr, & Greenlee, J. J. (2008). Identification of a heritable polymorphism in bovine PRNP associated with genetic transmissible spongiform encephalopathy: evidence of heritable BSE. PloS one, 3(8), e2912. https://doi.org/10.1371/journal.pone.0002912.

Madilindi, M. A., Banga, C. B., Bhebhe, E., Sanarana, Y. P., Nxumalo, K. S., Taela, M. G., Magagula, B. S., & Mapholi, N. O. (2020). Genetic diversity and relationships among three Southern African Nguni cattle populations. Tropical animal health and production, 52(2), 753–762. https://doi.org/10.1007/s11250-019-02066-y.

Maghsoodi, S. M., MIRAEI, A. S. R., Banabazi, M. H., & MEHRABANI, Y. H. (2011). Polymorphism of Prion Protein Gene (PRNP) in Iranian Holstein and two local cattle populations (Golpayegani and Sistani) of Iran, 9(2), 115-119.

Msalya, G., Shimogiri, T., Ohno, S., Okamoto, S., Kawabe, K., Minezawa, M., & Maeda, Y. (2011). Evaluation of PRNP expression based on genotypes and alleles of two indel loci in the medulla oblongata of Japanese Black and Japanese Brown cattle. PloS one, 6(5), e18787. https://doi.org/10.1371/journal.pone.0018787.

Murdoch, B. M., Clawson, M. L., Yue, S., Basu, U., McKay, S., Settles, M., Capoferri, R., Laegreid, W. W., Williams, J. L., & Moore, S. S. (2010). PRNP haplotype associated with classical BSE incidence in European Holstein cattle. PloS one, 5(9), e12786. https://doi.org/10.1371/journal.pone.0012786.

Murdoch, B. M., & Murdoch, G. K. (2015). Genetics of Prion Disease in Cattle. Bioinformatics and biology insights, 9(Suppl 4), 1–10. https://doi.org/10.4137/BBI.S29678

Peden, A. H., Head, M. W., Ritchie, D. L., Bell, J. E., & Ironside, J. W. (2004). Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet (London, England), 364(9433), 527–529. https://doi.org/10.1016/S0140-6736(04)16811-6.

Prusiner S. B. (1998). Prions. Proceedings of the National Academy of Sciences of the United States of America, 95(23), 13363–13383. https://doi.org/10.1073/pnas.95.23.13363.

Prusiner S. B. (2004). Detecting mad cow disease. Scientific American, 291(1), 86–93. https://doi.org/10.1038/scientificamerican0704-86.

Sander, P., Hamann, H., Drögemüller, C., Kashkevich, K., Schiebel, K., & Leeb, T. (2005). Bovine prion protein gene (PRNP) promoter polymorphisms modulate PRNP expression and may be responsible for differences in bovine spongiform encephalopathy susceptibility. The Journal of biological chemistry, 280(45), 37408–37414. https://doi.org/10.1074/jbc.M506361200.

Teferedegn, E. Y., Can, H., Erkunt Alak, S., & Ün, C. (2023). Low frequency of protective variants at regulatory region of PRNP gene indicating the genetically high risk of BSE in Ethiopian Bos indicus and Bos taurus africanus. Animal biotechnology, 34(6), 1931–1936. https://doi.org/10.1080/10495398.2022.2058007.

Un, C., Oztabak, K., Ozdemir, N., Tesfaye, D., Mengi, A., & Schellander, K. (2008). Detection of bovine spongiform encephalopathy-related prion protein gene promoter polymorphisms in local Turkish cattle. Biochemical genetics, 46(11-12), 820–827. https://doi.org/10.1007/s10528-008-9196-0

Vaccari, G., Petraroli, R., Agrimi, U., Eleni, C., Perfetti, M. G., Di Bari, M. A., Morelli, L., Ligios, C., Busani, L., Nonno, R., & Di Guardo, G. (2001). PrP genotype in Sarda breed sheep and its relevance to scrapie. Brief report. Archives of virology, 146(10), 2029–2037. https://doi.org/10.1007/s007050170050.

Yang, Q., Zhang, S., Liu, L., Lei, C., Qi, X., Lin, F., Qu, W., Qi, X., Liu, J., Wang, R., Chen, H., & Lan, X. (2018). The evaluation of 23-bp and 12-bp insertion/deletion within the PRNP gene and their effects on growth traits in healthy Chinese native cattle breeds. Journal of Applied Animal Research, 46(1), 505–511. https://doi.org/10.1080/09712119.2017.1348950.

Yaman, Y., & Ün, C. (2017). Genetic resistance to prion diseases. In Prion-An Overview. IntechOpen. Available from: http://dx.doi.org/10.5772/66916.

Yeh, F. C., Yang, R. C., Boyle, T. B. J., Ye, Z. H., & Mao, J. X. (2000). POPGENE 32, Microsoft Windows-based software for population genetic analysis (version 1.32). Molecular Biology and Biotechnology Centre, University of Alberta, Alberta, Canada.

Zhao, H., Wang, X. Y., Zou, W., & Zhang, Y. P. (2010). Prion protein gene (PRNP) polymorphisms in native Chinese cattle. Genome, 53(2), 138–145. https://doi.org/10.1139/g09-087