Prion protein polymorphisms in the Slovenian red deer (*Cervus elaphus*) population

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Introduction

Chronic Wasting Disease (CWD) was first discovered in European cervids in 2016. It was confirmed in reindeer (*Rangifer tarandus tarandus*), moose (*Alces alces*) and red deer (*Cervus elaphus*). Although official monitoring for CWD was only carried out in Slovenia in 2006 and 2007, we collected and examined 190 brains from red deer and 2 brains from Canadian deer (*Cervus canadensis*) between 2002 and 2021. All samples were negative. In the Slovenian red deer population, about 8000 animals are hunted annually. We determined the genotype of the prion protein gene (*PRNP*) in 24 red deer from different regions of Slovenia to determine whether PRNP polymorphisms associated with susceptibility to CWD are present in Slovenian red deer.

Material and methods

CWD monitoring

Samples were tested with rapid post-mortem test (Prionics® Check Western, Enfer or IDEXX), histopathology and in most cases with immunohistochemistry.

PRNP genotyping

Genomic DNA was isolated from the muscles of 24 animals (Wizard DNA extraction protocol - Promega).

The *PRNP*, including the whole open reading frame, was amplified by PCR (primers: SPrP-1, SPrP-2 (Gombojav *et al* 2003)).

The purified PCR products were sequenced by Sanger sequencing, the sequences were analysed in Chromas and aligned using the BLAST algorithm for species identification, Clustal Omega was used for sequence alignment.

Results

Table: The results of *PRNP* genotyping in red deer collected in different regions of Slovenia. Samples were from hunted healthy animals or animals found dead.

codon position	15	21	59	63	78	79	96	98	132	136	168	208	225	226	247	
animal No																region
1	VV (gtg/gtg)	VV (gtc/gtc)	GG	PP	QQ (cag/cag)	PP	GG	TT (acc/acc)	MM (atg/atg)	AA (gct/gct)	PP	MM	SS	**QQ (cag/cag)**	II	Prekmurje
2	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			**TA (acc/gcc)**	MM (atg/atg)	AA (gct/gct)				**QQ (cag/cag)**		Prekmurje
3	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gct)				**QE (cag/gag)**		Prekmurje
4	VV (gtg/gtg)	VV (gtc/gtc)			QQ (caa/caa)			TT (acc/acc)	MM (atg/atg)	AA (gct/gct)				**QQ (cag/cag)**		Prekmurje
5	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			**TA (acc/gcc)**	MM (atg/atg)	AA (gct/gct)				**QQ (cag/cag)**		Notranjska
6	VV (gtg/gtg)	VV (gtc/gtt)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gct)				**QE (cag/gag)**		Notranjska
7	VV (gtg/gtg)	VV (gtc/gtt)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gcc)				EE (gag/gag)		Notranjska
8	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gct/gct)				**QQ (cag/cag)**		Prekmurje
9	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gct)				**QE (cag/gag)**		Prekmurje
10	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gct)				**QE (cag/gag)**		Prekmurje
11	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gct)				**QE (cag/gag)**		Prekmurje
12	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			**TA (acc/gcc)**	MM (atg/atg)	AA (gcc/gct)				**QE (cag/gag)**		Kočevje
13	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gct)				**QE (cag/gag)**		Kočevje
14	VV (gtg/gtg)	VV (gtc/gtt)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gcc)				EE (gag/gag)		Kočevje
15	VV (gtg/gtg)	VV (gtc/gtt)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gcc)				EE (gag/gag)		Kočevje
16	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gcc)				EE (gag/gag)		Štajerska
17	VV (gtg/gtg)	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gct/gct)				**QQ (cag/cag)**		Prekmurje
2 - 2002	VV (gtg/gtg)	VV (gtc/gtt)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gcc)				EE (gag/gag)		
20	/	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gct)				**QE (cag/gag)**		
21	/	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gcc)				EE (gag/gag)		
22	/	VV (gtc/gtt)			QQ (cag/cag)			TT (acc/acc)	**ML (atg/ttg)**	AA (gcc/gcc)				EE (gag/gag)		
23	/	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gcc)				EE (gag/gag)		
24	/	VV (gtc/gtt)			QQ(cag/caa)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gct)				**QE (cag/gag)**		
25	/	VV (gtc/gtc)			QQ (cag/cag)			TT (acc/acc)	MM (atg/atg)	AA (gcc/gcc)				EE (gag/gag)		
aminoacids	VV (18); ND (6)	VV	GG	PP	QQ	PP	GG	**TT (21);** TA (3)	**MM (23);** **ML (1)**	AA	PP	MM	SS	**QE (9);** **QQ (6);** **EE (9)**	II	

Black - wild type; in colors - mutation; regular - silent mutation; bold - mutation causing change in aminoacid (AA); green - homozygous; orange - heterozygous; red - AA position where mutation was observed (ND - not determined).

Conclusions

Although only a small number of animals were genotyped, nucleotide variants at six *PRNP* codons were identified in these animals from the Slovenian red deer population. Of these six polymorphic codons, three were synonymous substitutions at AA positions 21, 78 and 136 and three were non-synonymous substitutions: (AA positions T(threonine)98A(alanine), M(methionine)132L(leucine) and Q(glutamine)226E(glutamate)). No new alleles were detected.

Our results suggest that the Slovenian red deer population is heterogeneous with respect to CWD genotypes at AA position 226, which may influence susceptibility to CWD. Q226 or E226 was present in red deer from all investigated regions. CWD may occur in Slovenian red deer, however, genotypes considered more resistant to CWD at position 226 are present in our red deer population.