Estimating sequence diversity of prion protein gene (*PRNP*) in Portuguese populations of two cervid species: Red deer and Fallow deer

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ABSTRACT

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INTRODUTION

Chronic Wasting Disease (CWD) belongs to the family of Transmissible Spongiform Encephalopathies (TSEs), specific to cervids, and characterized by an infectious, misfolding of the prion protein (PrP^{C}) into a protease-resistant form (PrP^{res}). Originated in North America and widespread in USA and Canada, the first case of CWD appeared in Europe in 2016 in a free-ranging reindeer in Norway and further cases were later reported in red deer and moose, ultimately reaching other countries like Finland and Sweden (Vikøren et al 2019; Arifin et al 2021). The study of susceptibility/resistance of cervids to CWD is essential to define its risk of dissemination/development as well as its potential as prion reservoir. The estimation of genetic variability in the prion protein (*PRNP*) gene is one of the methodologies used to predict that certain populations are less susceptible to infection than others (Haworth et al 2021). The analysis of the *PRNP* diversity in European cervid species is still limited but some studies carried out in Britain, Norway and Sweden, showed that some *PRNP* genetic variations can be related with CWD susceptibility/resistance. Genetics studies, in Europe and Portugal, to evaluate the variation of *PRNP* gene in cervid populations are of extreme importance in order to estimate potential susceptibility populations to emerging CWD and informing risk assessment and control/surveillance strategies.

MATERIAL AND METHODS

Samples
- ✓ Total Number of Samples 134 animals
- ✓ Red deer (*Cervus elaphus*) - 57
- ✓ Fallow deer (*Dama dama*) - 77

Genomic DNA Extraction
- ✓ Frozen Muscle and Lymphoid tissues
- ✓ NZY Tissue gDNA Isolation kit (NZYTech, Lda. - Genes and Enzymes)

PCR Amplification
- ✓ Full exon 3 of *PRNP* gene (771 bp)
- ✓ Primers F223-ACACCCTCTTTATTTTGCAG and R224-AGAAGATAATGAAAACAGGAAG)

Sanger Sequencing and Analysis
- ✓ Eurofins Genomics GmbH in Germany
- ✓ SnapGene Viewer v. 5.1.5
- ✓ Unipro UGENE v. 40.0
- ✓ Jalview 2.11.1.4

FINDINGS

I. The comparison of coding region *PRNP* gene (771 bp) and protein sequence - PrP^{C} (256 aa) showed:
1. Red deer (57 samples) - high conservation in protein sequence;
2. Fallow deer (77 samples) - no sequence diversity was found corresponding to the red deer haplotype T_{98}-E_{226} (TE) with an amino acid variation in codon 138, asparagine (N) for serine (S) (highlighted in Figure 2).

II. Presence of the terminal signals and the octorepeat domain (5 peptide repeats with 3 octapeptides of PHGGGWGQ flanked by 2 nonapeptides of P(Q/H)GGGWGQ) (highlighted in Figure 2).

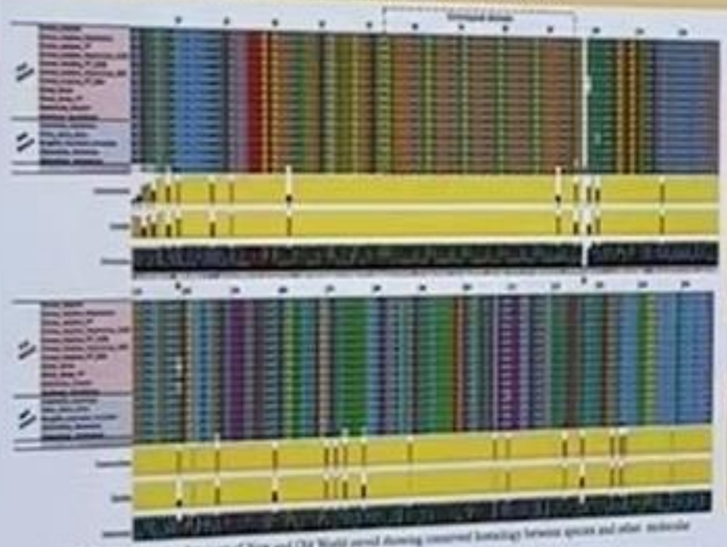

Figure 2. Protein sequence alignment of New and Old World cervid showing conserved homology between species and other molecular conserved features.

III. Three single-nucleotide polymorphisms (SNPs) were identified:
1. One synonymous SNPs - position 408 (codon 136) (gcT/gcC);
2. Two non-synonymous mutations: position 292 (Acc/Gcc)/ T98A and position 676 (Cag/Gag)/ Q226E (Table 1 and 2).

IV. The synonymous mutation at position 408 (codon 136) showed to be linked to the non-synonymous mutations at position 676 (codon 226).

Figure 1. Map of the southeast of Portugal (Beira Baixa district) showing the geographic locations where different samples were obtained.

Table 1. Amino acid variation within the exon 3 and haplotype frequencies of cervid *PRNP* in red deer population

Table 2. Genotype frequencies of *PRNP* polymorphisms in Portuguese red deer populations

CONCLUSION

In summary, is our opinion that multi-disciplinary approaches including genotyping, PrP^{res} detection, identification of risk factors and others, are of great importance to evaluate the risk of occurrence of CWD in European and more specific in Iberian Peninsula. In this way a synergistic collaborative project (Project 029947ICA&T 02/SAICT/2017-SAICT) was established between the University of Trás-os-Montes and Alto Douro (UTAD), the National Institute for Agricultural and Veterinary Research (INIAV) and the Polytechnic Institute of Castelo Branco (IPCB) with the aim of evaluating the risk of a potential occurrence of CWD in cervid Portuguese populations.

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