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WAVES

Assessing Chronic Wasting Disease risk in Portugal

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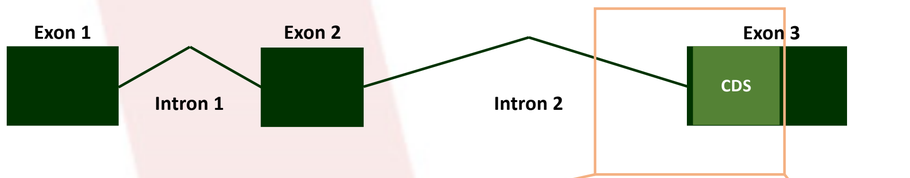
INTRODUCTION

The identification of Chronic Wasting Disease (CWD) in Norway guides the possibility that cervids population in Europe could be at risk for Transmissible Spongiform Encephalopathies (TSEs) and can represent a potential prion reservoir, as it occurs in other diseases, menacing the livestock and public health. A collaborative project (Project 029947IC&T 02/SAICT/2017-SAICT) was established between the University of Trás-os-Montes e 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 Portugal (Figure 1). This is a synergistic collaboration as both UTAD and IPCB are located in areas with a closer contact with the cervid population and INIAV is the national reference laboratory for animal TSEs.

In Portugal there are 3 species of cervids: red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*); and the probability of these species being exposed to CWD is not negligible, as Portugal had a high prevalence of Bovine Spongiform Encephalopathy (BSE) (Orge *et al.*, 2015) and an outbreak of classical scrapie was also detected in a context of atypical scrapie, in sheep and goats (Orge *et al.*, 2010). Due to the extensive grazing areas shared by the population of wild ruminants and herds of sheep and goats, actual contact with prions is likely to occur.

MATERIAL AND METHODS

By now, a total of 250 samples were obtained. Fifty tissue samples from Portuguese red deer and fallow deer were already used for DNA extraction. Specific primers designed to amplify *prnp* gene were used (Figure 2). All the PCR products were already obtained with success.



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ctttaagtgatttttacatgggcatatgatgctgacaccctctttatttttgcagATAAGTCATC**ATGGT**
GAAAAGCCACATAGGCAGCTGGATCCTAGTTCTCTTTGTGGCCATGTGGAGTGACGTCGGCCTCTGCAA
GAAGCGACCAAAACCTGGAGGAGGATGGAACACTGGGGGGAGCCGATACCCGGGACAGGGAAGTCCTGG
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TCATGGAGGTGGCTGGGGTCAGCCCCATGGTGGTGGCTGGGGACAGCCACATGGTGGTGGAGGCTGGGG
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AGCTGCTGCAGCTGGAGCAGTGGTAGGGGGCCTCGGTGGCTACATGCTGGGAAGTGCCATGAGCAGGCC
TCTTATACATTTTGGCAATGACTATGAGGACCGTTACTATCGTGAAAACATGTACCGTTACCCCAACCA
AGTGTACTACAGGCCAGTGGATCAGTATAATAACCAGAACACCTTTGTGCATGACTGTGTCAACATCAC
AGTCAAGCAACACACAGTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGATGAT
GGAGCGAGTTGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAATCCGAGGCTTATTACCAAAGAGG
GGCAAGTGTGATCCTCTTCTCCTCCCCTCCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGT
AGGATAGGGGCAACCTTCCTGTTTTCATTATCTTCT

Figure 2 – Illustrative scheme of the *prnp* gene in *Cervus elaphus*, composed of 3 exons and 2 introns and partial sequence of *prnp* gene (GenBank accession: FJ590751.1), including part of the intron 2 in lowercase, the exon 3 in uppercase and the coding sequence (CDS), bolded and highlighted in grey, within the exon 3. For the amplification of this region, the primers forward (light blue) and reverse (yellow) were used.

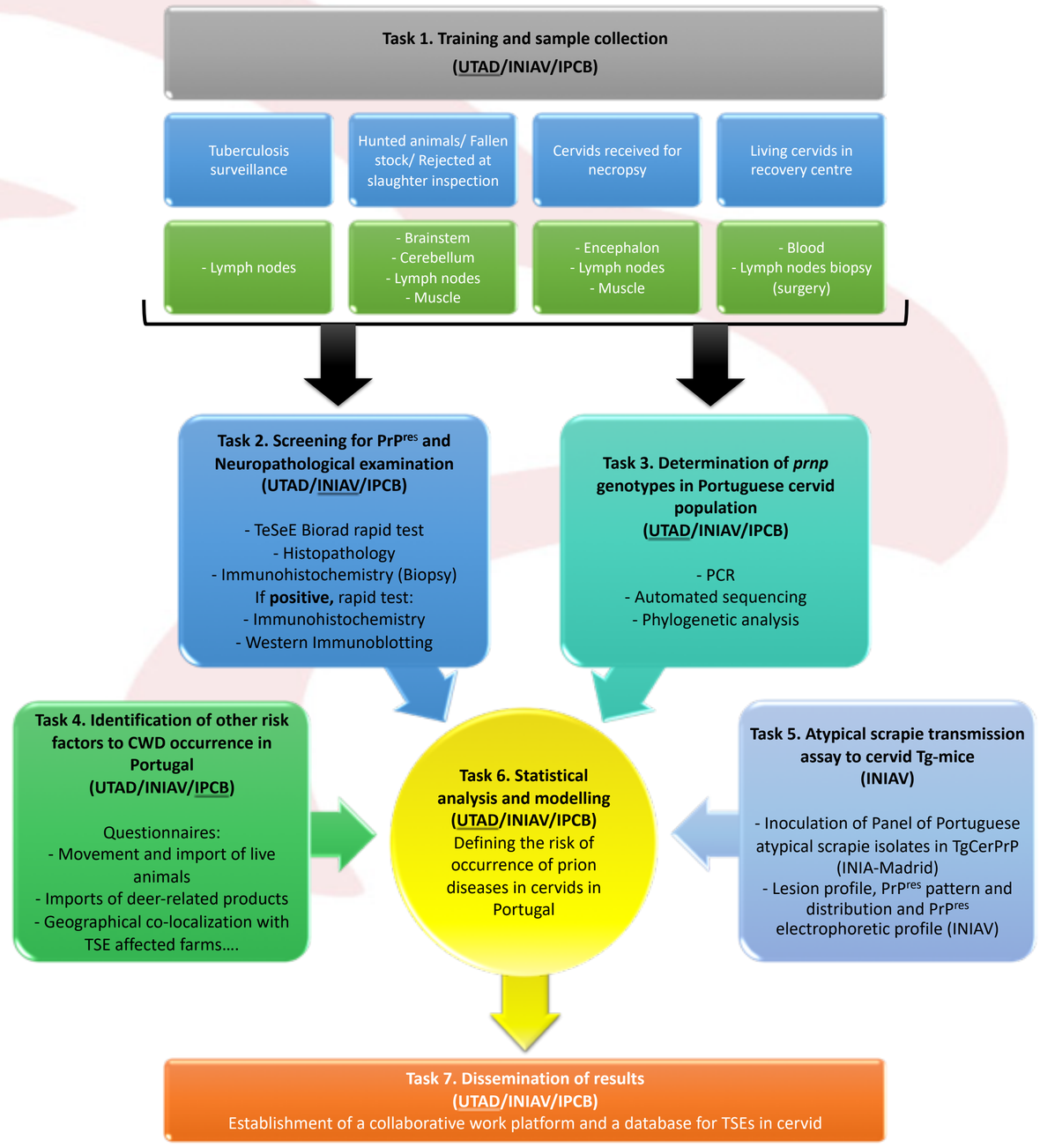

Figure 1 – Diagram of tasks.

CONCLUSION

The sequencing analysis of this region in all the animals will allow us to find and characterize polymorphic sites and to genotype them and compare with the data from other countries. 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 susceptibility/resistance to prion diseases are influenced by polymorphisms in the *prnp* gene, so, both genotypic characterization in cervids and PrP^{res} survey will contribute to delineate the dissemination risk of CWD in Portugal.

Some mutations in the CDS region, which promote changes in the amino acid sequence, seems to influence the phenotype of individuals. Likewise, intronic regions are much less conserved sequences and are often associated with gene regulation, because they may contain regulatory sequences (Vaz-Drago *et al.*, 2017), such as enhancers, RNA genes non-coding, RNA binding proteins (RBP) binding sites, splicing sites, which may promote alternative splicing or alteration in their expression. McCormark *et al.* (2002) demonstrated that variations in the intronic region of the human *PRNP* gene may influence human prion diseases. In this context, once the transcription in mammals is very dependent on these regulatory regions, it is interesting to study polymorphisms not only in the CDS region but also of non-coding regions.

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