Analysis of the PrP gene at codon 136 in Tunisian sheep using sequencing method

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Abstract: Scrapie is a degenerative disease of the nervous system and is a subject of international concern. Several studies have shown that in sheep, susceptibility to scrapie is mainly influenced by the polymorphism of the PrP gene. There is indeed a strong link between different forms of PrP gene (alleles) and susceptibility to scrapie. In Tunisia, no study has focused on the characterization of Tunisian sheep population on genetic resistance to scrapie. It is in this context that the present study is aimed to assess the frequency of alleles of the PrP gene in the most common breeds of sheep in Tunisia. The DNA of 24 animals belonging to two main Tunisian sheep breeds: the Barbarine (12 individuals) and fine tail west (12 individuals) was extracted from whole blood and amplified by PCR using primers flanking the codon 136 of the PrP gene. The amplicons were sequenced to determine the genotype of each animal. The amplification was generated in all animals, an amplicon size of 120 bp. All animals studied were found homozygous (A / A) at codon 136. AA136 genotype is associated with greater resistance to scrapie, while VV136 genotype is associated with greater susceptibility to disease. Based on various studies, showing the predominance of three codons 136, 154 and 171 in the mechanisms of resistance and susceptibility to scrapie, we have developed a protocol for the analysis of codon 136 of the PrP gene.
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1.Introduction

Small ruminants, including sheep, are often affected by a wide range of diseases, which has socio-economic constraints causing huge losses to both the breeders and the national economy. Scrapie disease is a matter of international concern. Several studies have shown that in sheep, susceptibility to scrapie is mainly influenced by the polymorphism of the PrP gene (Vaccari et al., 2001; Drogemuller et al., 2004). In sheep, the PrP gene is located on chromosome 13 and consists of three exons and two introns and encodes a protein of 256 amino acids (Goldmann et al., 2005). Polymorphism at codons 136, 154 and 171 respectively encoding alanine (A) or valine (V), arginine (R) or histidine (H) and R, H or glutamine (Q) is known for be closely linked to the degree of sensitivity or resistance to scrapie (Tongue et al., 2004; Gama et al., 2006). Different countries have already implemented programs control and eradication of scrapie based on the use of reproductive genetically resistant to the disease. Despite the importance of the sheep breeding sector in the Tunisian agricultural economy, no study has focused on the analysis of codons 136, 154 and 171 of the PrP gene.

The objective of this work is the study of the PrP gene polymorphism at codon 136 in animals belonging to the two most common native Tunisian sheep breeds representing the major sheep population, involved in the mechanisms of resistance and susceptibility to scrapie.

2.Materials and methods

Samples collection and DNA extraction:

Samples were randomly collected from 24 animals; 12 animals from the Barbarine (B) breed and 12 animals from the Western thin tail (W), six males and six females were sampled from each breed, Blood samples from both sexes of pure B and W breeds were collected from the jugular vein in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes and kept at -20°C until the isolation of total DNA. DNA extraction was carried out using a genomic purification kit (blood DNA preparation kit, Jena Bioscience). In order to improve the quality and quantity of extracted DNA, some modifications to the standard protocol were conducted. The red blood cell lyses step was followed by additional washes by adding 900 µl of bidistilled sterilized water to the sample, vortexing vigorously for 5 min, centrifuging at 13000 rotations per minute for 5 min and removing the supernatant. This stage was repeated until obtaining a clean pellet rid of any trace of hemoglobin. The DNA hydration time was prolonged to three days and conducted in dark at room temperature. According to the kit manual, a 300 µl sample of whole blood yields 10 to 20 µg of DNA.

DNA quality and quantity were controlled using analysis on agarose gels and spectrophotometry.

PCR conditions:

PCR amplifications were performed in 50 µl reaction mixtures containing 30 ng of genomic DNA, 0.8 µM of each primer, 300 µM of dNTP (dNTP Mix, Jena Bioscience), 3 mM of MgCl2, 1.25 unit of Taq DNA polymerase (ULTRATOOLS DNA Polymerase, Biotools) and 5 µl of 10X Taq DNA polymerase buffer. In order to detect any DNA contamination, control reactions were set up without genomic DNA. Amplifications were performed using a thermal cycler (Eppendorf, Mastercycler gradient) programmed for 30 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 2 min. An initial denaturation step of 2 min at 94°C and a final extension step of 10 min at 72°C were included in the first and last cycles, respectively. PCR reactions were carried out using a forward and a reverse primer flanking the 136 codon position (primer 136F: 5'-ATG AAG CAT GTG GCA GGA GC-3'; primer 136R: 5′ -ACG GTC CTC ATA GTC ATT GCC - 3′ (L'Homme et al. 2008).

DNA sequencing and analysis:

PCR products were purified using PCR Purification Kit (Jena Bioscience) and eluted in 30 µl dd-water, controlled in a 1.2% agarose gel containing ethidium bromide in Tris-borate EDTA buffer and visualized under UV transillumination. Sequencing of the PCR products was carried out by an authorized laboratory in an ABI Prism 310 (Applied BioSystems). Each sample was sequenced independently using both forward and reverse primers. The sequenced fragment contains the sequence tgggaagtgccatgagca or tgggaagtgtcatgagca on whether the codon 136 encodes the alanine or valine respectively (deduced by complementarity with the probes used by Van Pouke et al., (2005)).

3.Results and discussion

DNA from 24 animals was purified from whole blood samples, submitted to PCR and the amplicons' sequence were determined by Sanger sequencing in order to establish each animal's genotype for the codon 136 of the PrP gene. The amplification was generated in all individuals, an amplicon size of 120 bp (figure 1).

All analyzed animals were A/A homozygous for the 136 codon. The genotype AA136 is associated with the highest resistance to scrapie, whereas the genotype VV136 is associated with the highest susceptibility to disease. Scrapie is a spongiform encephalopathy similar to the BSE in cattle. If the nature of the pathogen and routes of transmission of the disease are not known with certainty, however, the existence of a genetic control of susceptibility to scrapie is well established (Hunter et al., 1996; Goldmann et al., 1994). Based on various studies, showing the predominance of three codons 136, 154, 171 in the mechanisms sensitivity resistance to scrapie (Laplanche et al., 1993a 1993b; Clouscard et al., 1995; Elsen et al., 1999), we have developed for the first time in Tunisia, a test for the analysis of the PrP gene at codon 136. We will soon expand genotyping to two other codons (154 and 171) of the same gene.

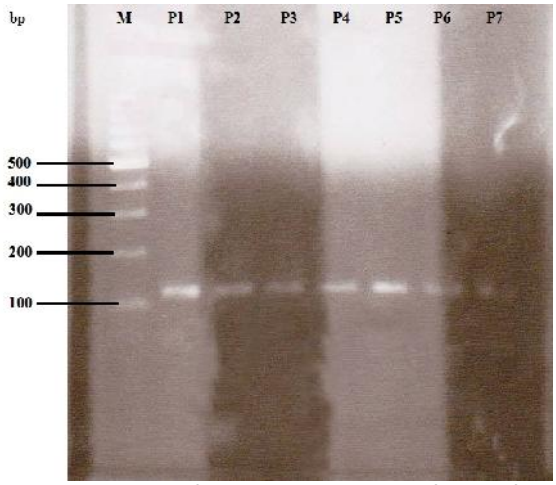

Figure 1. Amplification product profiles of 7 Tunisian sheep individuals generated for the codon 136 of the PrP gene. M, 100 bp DNA ladder (100 bp to 1 Kb); P1, P2 and P7, DNA sampled from Barbarine rams; P4 and P6, DNA sampled from Barbarine ewes; P3, DNA sampled from Western thin tail ram; P5, DNA sampled from Western thin tail ewe.

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