PrP gene polymorphism in Tunisian sheep using sequencing method

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Abstract: Scrapie is a fatal transmissible spongiform encephalopathy (TSE). Several studies have shown that in sheep, susceptibility to scrapie is mainly influenced by the polymorphism of the PrP gene. The identification of PrP genotypes for scrapie is very important for every country in order to develop and implement scrapie breeding program. In Tunisia, no study has focused on the characterization of Tunisian sheep population on genetic resistance to scrapie. The aim of this study was to assess the PrP polymorphisms in a total of 48 healthy sheep from two Tunisian native sheep breeds. There were three alleles and three genotypes observed based on codons 136, 154 and 171 of PrP gene. The ARQ allele was predominant (72.92%) while the ARH allele was found at low frequency in the global population (8.33%).
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1. Introduction

Scrapie is a prion disease that reaches small ruminants. This disease is a prototypical transmissible spongiform encephalopathy (TSE) (Parry, 1983; Wood et al., 1997). It is characterized by the appearance of behavioral disorders associated with central nervous system and is always fatal. In sheep, the genotype at codons 136, 154 and 171 of the PrP gene determines susceptibility or resistance to disease potential. Typing the PrP gene can be carried out on sheep of all ages and it is a valuable tool to select animals with natural resistance to scrapie. In sheep, the PrP gene is located on chromosome 13 (OAR13). It exists five different alleles (ARQ, VRQ, AHQ, ARR and ARH), leading to 15 different genotypes (Belt et al., 1995; Bossers et al., 1996; Hunter et al., 1996).

In Tunisia, Sheep farming plays a vital role in food security. Indeed, this sector provides more than 48% of the country's total red meat production (Mohamed-Brahmi et al., 2010). However, no program for the control and eradication of scrapie has been implemented in Tunisia.

The aim of the present investigation was to study the PrP gene polymorphism at codons 136, 154 and 171 in animals belonging to the two most common native Tunisian sheep breeds (the Barbarine (B) and the Western thin tail (W)).

2. Material and Methods

Samples collection, DNA extraction and polymerase chain reaction (PCR) amplification, DNA sequencing and statisticals analysis

Blood samples were collected on ethylenediaminetetraacetic acid (EDTA) tubes from the jugular vein were randomly collected from 48 animals. 12 males and 12 females were sampled from each breed. Blood samples were stored at -20°C until isolation of total DNA. DNA extraction was carried out using a genomic purification kit (blood DNA preparation kit, Jena Bioscience) with some modifications. 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 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 63°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 codons 136, 154 and 171 (sens primer: 5'-GGAGGCTGGGGTCAAGGT-3', and antisens primer: 5'-GGTGGTGGTGACTGTGTGTTG-3' (Van poucke et al., 2005).

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 DNA sequences were analyzed using the Sequencing Analysis Software Version 3.3 (Applied Biosystems, Foster City, CA, USA). $\chi 2$ test (to evaluate possible Hardy-Weinberg equilibrium), allele frequencies and genotype frequencies were carried out by using PopGene software (Yeh et al., 2000).

3. Results and discussions

DNA from 48 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 codons 136, 154 and 171 of the PrP gene. The amplification was generated in all individuals, an amplicon size of 315 bp (figure 1).

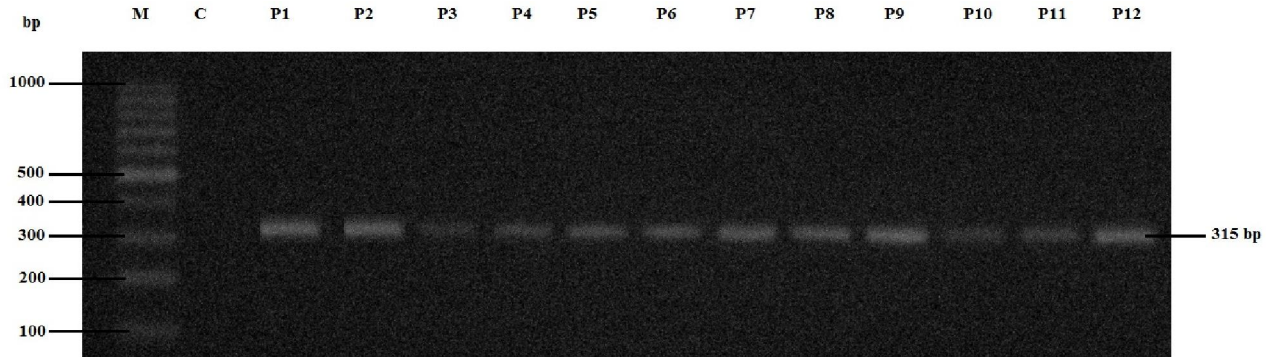

Figure 1. Amplification product profiles of 12 Tunisian sheep individuals generated for the PrP gene. M, 100 bp DNA ladder (100 bp to 1 Kb);C, P1→P6, DNA sampled from Barbarine animals; P7→P12, DNA sampled from Western thin tail animals.

All analyzed animals were A/A and R/R homozygous for the 136 and 154 codons respectively. Allele and Genotypic frequencies and Hardy-Weinberg equilibrium for codon 171 were calculated and are given in Tables 1 and 2. This population was monomorphic for PrP codons 136 and 154; while deviated from Hardy-Weinberg equilibrium ($P > 0.05$) for codon 17. Observed heterozygosity and expected heterozygoty for 171 locus were 0.5417 and 0.4262 respectively (Nei, 1973). The percentage of ARQ allele was 72.92%, this allele is associated with medium- high susceptibility to scrapie. The frequency of ARR allele was 18.75%. It is well known that animals carrying at least one ARR allele are semi-resistant to disease (Goldmann, 2008). In the presence of an ARR allele, the prion multiplication is very slow, it is limited to the nervous system, and the prion is not detectable before the onset of clinical signs. The VRQ allele was not detected in our sample. The VRQ allele has been associated with the highest risk to to develop scrapie. The classical form of the disease most frequently occurs in sheep with ARQ/VRQ, ARH/VRQ and VRQ/VRQ genotypes (Gonzalez et al., 2010).

In Tunisia, scrapie control breeding programs have not been established for scrapie. Based on various studies, showing the predominance of three codons 136, 154, 171 in the mechanisms sensitivity resistance to scrapie (Laplanche et al., 1993; Clouscard et al., 1995; Elsen et al., 1999), we have set up a protocol for the analysis of the PrP gene at codons 136, 154 and 171. This study showed that the Tunisian sheep flock nevertheless enjoys a pool of alleles (ARR) for an interesting selection to resistance to scrapie. However, this study needs to be completed in order to have a clear idea of various genotypes in the main Tunisian sheep breeds and to study their association with the genetic potential productivity of sheep.

Table 1. Allele frequencies at codon 171 in Barbarine breed (B), Western thin tail breed (W) and overall population

Allele	B	W	Overall population
Q	0.7917	0.6667	0.7292
R	0.1667	0.2083	0.1875
H	0.0417	0.1250	0.0833

Table 2. Observed (O), expected (E) Genotypic frequencies and chi-square test for Hardy-Weinberg equilibrium at codon 171.

Genotypes	O	E	$(O-E)^2/E$
(Q,Q)	22	25.4211	0.4604
(R, Q)	18	13.2632	1.6917
(R,R)	0	1.6105	1.6105
(H,Q)	8	5.8947	0.7519
(H,R)	0	1.5158	1.5158
(H,H)	0	0.2947	0.2947
chi-square ratio test for Hardy-Weinberg equilibrium:			
Chi-square: 6.325052			
Degree of freedom: 3			
Probability: 0.096823			

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