DNA polymorphism at the 154 codon of the prion protein gene in Tunisian sheep breeds using sequencing method

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Abstract: The present study was undertaken to investigate the PrP gene polymorphism at codon 154 in two major Tunisian sheep breeds, namely the Barbarine (B) and Western thin tail (W). It involved 24 animals, with 12 from each breed. Genomic DNA was extracted from whole blood samples and amplified by PCR using primers flanking the codon 154 of the PrP gene. The amplicons were sequenced to determine the genotype of each animal. Amplification was performed for all animals, generating an amplicon size of 180 bp. The findings revealed the prevalence of a homozygote genotype RR in the population. The heterozygote genotype RH was observed in only one animal belonging to the W breed. The homozygote genotype HH was not observed in both breeds.
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1.Introduction

Although the infectious nature of scrapie has long been recognized, the variability so far observed in the natural forms of scrapie suggests the presence of potential genetic links. In a previous study involving multiple ovine lines, Dickinson et al. (1968) reported that the samples produced distinct incubation periods and patterns, thus providing evidence for a Mendelian genetic determinism. They reported that incubation period (Scrapie Incubation Period) was controlled by a major autosomal gene, the SIP gene which has two alleles, namely sA (short incubation) and pA (prolonged incubation). Later, the SIP and PrP genes were identified as one and the same gene (Lantier et al., 1995).

Various studies have shown that there are major codons (136, 154 and 171) directly affecting susceptibility to scrapie, which helped distinguish resistant, susceptible, and moderately resistant animals (Goldman et al., 1994; Elsen et al., 1999). Given current knowledge, the ARR allele seems to offer a promising approach for the control and prevention of scrapie. Although several fundamental questions still remain unanswered (existence of healthy carriage, ARR universal resistance and atypical scrapie discovered in 1998, etc.), various control programs have emerged throughout the world, including Britain, France and Netherlands, based on the selection of resistant animals (ARR allele carriers). The present study was undertaken to investigate the PrP gene polymorphism at codon 154 in 24 sheep samples from two major Tunisian sheep breeds, Barbarine (B) and Western thin tail (W), in which codon 136 polymorphism has previously been explored (El-Hentati et al., 2013).

2.Materials and methods

A total of 24 blood samples were randomly collected on EDTA tubes from the jugular vein of both male (6) and female (6) sheep from each Tunisian breed (B, 12 sheep and W, 12 sheep). DNA extraction was performed using a genomic purification kit (blood DNA preparation kit, Jena Bioscience). The quality and quantity of extracted DNA were improved using a slightly modified version of the standard protocol. In brief, the quality and quantity of DNA were controlled by agarose gel and spectrophotometric analyses. PCR amplifications were carried out in 50 µl reaction mixtures consisting of 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. Control reactions were set up without genomic DNA to identify potential DNA contamination. Amplifications were conducted in a thermal cycler (Eppendorf, Mastercycler gradient) programmed for 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. The initial and final cycles included an initial denaturation step of 2 min at 94°C and a final extension step of 2 min at 72°C, respectively. A forward and a reverse primer flanking the 154

position (primer 154F: 5′ - ATG AAG CAT GTG GCA GGA GC-3’; primer 154R: (5’- CTG ATC CAC TGG TCT GTA GTA CAC - 3′) (L’Homme et al. 2008) were used in the PCR reactions. The PCR products were purified by a PCR Purification Kit (Jena Bioscience), 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. They were then sequenced by an ABI Prism 310 genetic analyzer (Applied BioSystems) at a local certified laboratory. Each sample was separately sequenced using both forward and reverse primers, and DNA sequences were analyzed by the Sequencing Analysis Software Version 3.3 (Applied Biosystems, Foster City, CA, USA).

3.Results and discussion

Amplification was performed for all samples, showing an amplicon size of 180 bp (figure 1). The findings revealed the predominance of the homozygote genotype RR in the sheep population (23 animals). The heterozygote genotype RH was observed in only one sheep, which belonged to the W breed (Table 1). The homozygote genotype HH was not observed in the sheep population under investigation.

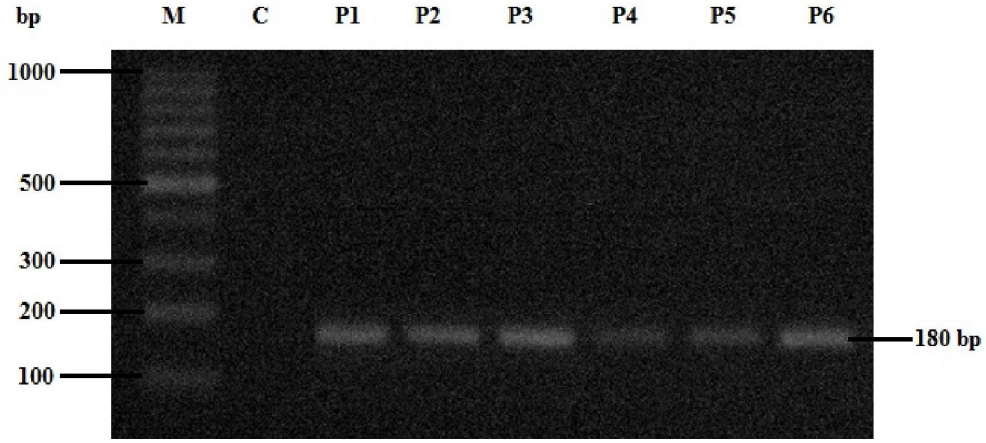

Figure 1. Amplification product profiles of six Tunisian sheep individuals generated for the PrP gene. M, 100 bp DNA ladder (100 bp to 1 Kb); C: control sample, P1→P3, DNA sampled from Barbarine animals; P4→P6, DNA sampled from Western thin tail animals.

Table 1. Allele Frequencies at codon 154 in Barbarine breed (B), Western thin tail breed (W), and overall population

Allele	B	W	Overall population
R	1	0.9583	0.9792
H	0	0.0417	0.0208

These data were consistent with observations in other studies. Guan et al. (2011) have, for instance, reported that the R allele was prevalent in Chinese Hu sheep with a frequency of 99.4%. Likewise, Acin et al. (2004) investigated the prion gene polymorphism in Spanish sheep and reported on the predominance of the R allele. The literature presents strong evidence for the variability of the PrP locus. Three codons are often reported to play a decisive role in susceptibility to scrapie: codons 136, 154 and 171. They determine the position of five amino acids, namely Alanine (A), Arginine (R), Histidine (H), glutamine (Q) and Valine (V), which control the sensitivity or resistance of the animal to scrapie. Different alleles encoding variants of the PrP protein are designated by three letters corresponding to amino acids position 136, 154 and 171. Thus, alleles Alanine in 136 and Arginine in 154 and 171 (codified combination ARR) are linked to the resistance of the animal to classical scrapie. Alleles Valine in 136, Arginine in 154 and Glutamine in 171 (combination codified VRQ), on the other hand, confer high susceptibility to scrapie. Homozygous sheep ARR/ARR never show clinical signs, and heterozygous ARR are rarely clinically affected (Andreoletti et al., 2000; Detwiler et al., 2000). Overall, this study provided preliminary information on the DNA polymorphism at the 154 codon of the prion protein gene in two major Tunisian sheep breeds, namely the Barbarine (B) and Western thin tail (W). The findings revealed the prevalence of the RR genotype. The results of this work, together with those previously reported by El-Hentati et al. (2013), provide a significant step towards understanding the genetic susceptibility of Tunisian sheep breeds to scrapie, an area of research that has long been in need of further exploration.

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