

Prion protein (PrP) gene polymorphism at codon 171 in Tunisian sheep breedsHaifa El-Hentati^{1,2,3}, Mohamed Ben Hamouda¹, Abderraouf Kenani², Ali Chriki³¹ Regional center of agricultural research and development on north-east, Po Box 122, 2090 Mornag, Institution of Agricultural research and higher education (IRESA), Tunisia² Laboratory of Biochemistry 05/UR/09-09, Faculty of Medicine of Monastir, University of Monastir, Tunisia³ Faculty of Sciences of Bizerta, 7021 Jarzouna, University of Carthage, Tunisiahaifa_ch@yahoo.fr

Abstract: Transmissible spongiform encephalopathies (TSE) are a complex group of fatal neurodegenerative diseases affecting humans and animals, of which scrapie is a prototype in sheep and goats. Although sheep scrapie has been known for a long time, considerable uncertainties still surround its occurrence, including its pathophysiological features, modes of action, and mechanisms of spread. With these concerns in mind, the present study was undertaken to sequence and analyze PrP gene polymorphism at codon 171 in two Tunisian sheep breeds (Barbarine and Western thin tail). The H allele was detected at a significantly lower frequency when compared to the Q and R alleles in both breeds. The findings also revealed three genotypes (QQ, RQ and HQ) in the overall population, with frequencies of 0.5%, 33.33%, and 16.67%, respectively.

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1.Introduction

Scrapie belongs to a group of diseases known as transmissible spongiform encephalopathies (TSE). Although scrapie was first reported by Leopoldt in 1750 (Leopoldt, 1750), the literature indicates the occurrence of the disease dates back to much earlier time periods (Bradley, 1997). Sheep susceptibility to scrapie has been attributed to various inoculation routes, with the oral route being the major, but not most efficient, route. Following inoculation, the scrapie agent undergoes a replication/propagation phase in lymphoreticular tissues, including tonsils, spleen, lymph nodes retropharyngeal, and mesenteric and peripheral nerve tissues. Several studies have, therefore, stipulated that as long as its spreads into the lymph nodes, the scrapie agent can reach many non-nervous tissues. The literature indicates that replication/propagation phase can last for several months before the agent reaches the brain (Detwiler and Baylis, 2003).

There is strong evidence in the literature that homozygous ARR sheep are totally resistant to scrapie and that heterozygous ARR sheep are partially resistant to the disease. Alleles VRQ, ARQ, ARH, AHQ correspond to decreasing sensitivity (ARH is very rare and usually equated with ARQ) (Tongue et al., 2004, Gama et al., 2006). Those differences in sensitivity will transcribe significantly in the number of cases according to the genotypes of the animals. In the absence of an efficient treatment against scrapie, recent research indicates that genetic selection based on polymorphisms at codons 136, 154 and 171 of the gene encoding the prion protein (PrP), which are

associated with susceptibility to the disease, is the route of choice for scrapie eradication.

The selection of genetically resistant sheep began in Europe, including the Netherlands in 1998, UK in 2000, and France in 2001 (François et al., 2003). In classical scrapie, genetic selection is currently based on eliminating the VRQ allele and increasing the frequency of the ARR allele. Although several scrapie control and eradication programs have emerged worldwide for the selection of genetically resistant or tolerant genotypes, no study has so far been performed to explore the PrP variability of Tunisian sheep breeds. Tunisian flock consists mainly of the Barbarine (B) and Western thin tail (W) breeds, representing 60.3 and 34.6 of the Tunisian sheep population, respectively (Rekik et al., 2005). The present study aimed to sequence and analyze PrP gene polymorphisms at codon 171 in the Tunisian B and W breeds.

2.Materials and methods

A total of 24 blood samples were randomly collected on EDTA tubes from the jugular vein of animals of both sexes belonging to two different breeds (B) (12 sheep) and (W) (12 sheep); six males and six females were sampled from each breed. DNA extraction was carried out using a genomic purification kit (blood DNA preparation kit, Jena Bioscience). A slightly modified version of the standard protocol was used to improve the quality and quantity of extracted DNA. In brief, DNA quality and quantity were controlled using agarose gel and spectrophotometric analyses.

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. Control reactions without genomic DNA were set up to detect potential DNA contamination. Amplifications were performed using a thermal cycler (Eppendorf, Mastercycler gradient) programmed for 40 cycles of denaturation at 94°C for 1 min, annealing at 59°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 2 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 171 position (primer 171F: 5' - CGT GAA AAC ATG TAC CGT TAC CCC-3'; primer 171R: (5'- GGT GAC TGT GTG TTG CTT GAC TG - 3') (L'Homme et al. 2008). PCR products were purified using 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. The PCR products were sequenced at an authorized laboratory using an ABI Prism 310 genetic analyzer (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).

3.Results and discussion

Amplification was generated in all individuals, with an amplicon size of 120 bp (figure 1).

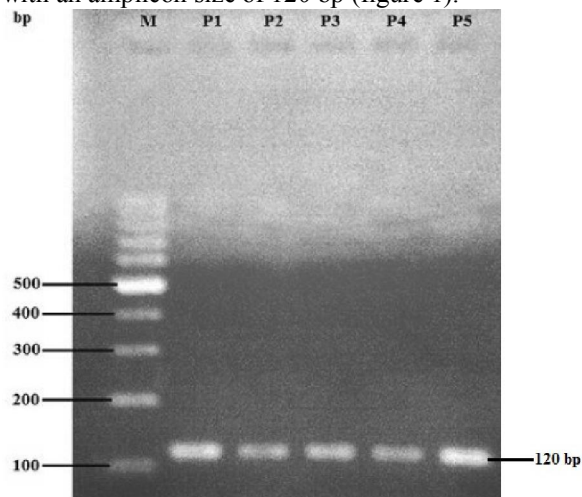


Figure 1 - Amplification product profiles of 5 Tunisian sheep individuals generated for codon 171 of the PrP gene. M, 100 bp DNA ladder (100 bp to 1 Kb); P1→P5, DNA sampled from Barbarine rams.

The findings revealed that the Q, R and H allele frequencies for all the studied individuals were 75%, 16.67%, and 8.33%, respectively. These allelic frequencies were, however, noted to vary between breeds (table 1). No significant differences were observed between ewes and rams ($p > 0.05$). In fact, most of the studies focusing on the genotyping of codon 171 at the PrP gene reported on the predominance of the Q allele in the studied population, with the H allele being less frequent. This prevalence of the Q allele was, for instance, shown in the works of Youngs et al., (1997), Kutzer et al, (2002) and Sipos et al, (2002) reporting on the percentages recorded for the Q, R and H alleles in Hampshire breed from Oklahoma (72.14, 1.26 and 26.6), Friesian Milk S. sheep from Germany (90.45, 8.9 and 0.65), and Tyrolean mountain sheep from Austria (74.3, 25.8 and 0), respectively.

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

Allele	B	W	Overall population
Q	70.83	79.17	75
R	25	8.33	16.67
H	4.17	12.5	8.33

The findings revealed that while the RR, HR or HH genotypes were absent, the QQ genotype was present in a higher frequency when compared to the other genotypes (0.5%) (table 2). In fact, these genotypes are often reported to have a very low to none frequency in sheep breeds. Sipos et al. (2002) showed that the RR genotype was absent in Tyrolean stone sheep in Austria. The HR genotype proved absent in several species, including Montadale, Dorset, Hampshire and Suffolk in Oklahoma (De Silva et al, 2003); Nolana, Friesian Milk S., and Bleu du Maine in Germany (Kutzer et al. , 2002); and Carylthian, Tyrolean stone, Forest, and Tyrolean mountain in Austria (Sipos et al., 2002). The HH genotype was, on the other hand, found absent in 120 Iranian sheep (Salami et al., 2011). The likelihood ratio test for Hardy-Weinberg equilibrium at codon 171 showed that the population deviated from the Hardy-Weinberg equilibrium ($P > 0.05$).

In a previous study, the authors have shown that all studied animals were A/A homozygous for the 136 codon (El-Hentati et al., 2013). Although the animal sample size investigated in the present study is relatively small to study the susceptibility/resistance of the Tunisian sheep population to scrapie, it represents the first attempt to explore PrP gene polymorphism at codon 171 in Tunisian sheep breeds. Further studies using larger samples would be needed to confirm and extend these findings. In fact, the

planning for an effective control program to fight scrapie in Tunisian sheep breeds will not be possible unless the sensitivity of different genotypes to natural or experimental scrapie is elucidated in a sufficient number of animals.

Table 2. Genotypic percentages observed at codon 171 in Barbarine breed (B), Western thin tail breed (W), and overall population

Genotypes	B	W	Overall population
(Q,Q)	41.66	58.33	0.5
(R,Q)	0.5	16.67	33.33
(R,R)	0	0	0
(H,Q)	8.33	25	16.67
(H,R)	0	0	0
(H,H)	0	0	0

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