ABSTRACT

This study was carried out for the identification and characterization of the genetic resistance against scrapie disease in Tunisian Sheep population. The study was conducted on two main Tunisian sheep breed viz Barbarine and Western thin tail. Furthermore, Allele and genotype frequency for three codons i.e. 136, 154 and 171 of the PrP gene was checked in these two breeds. Results of the study revealed that genotype ARQ / ARQ found in highest frequency (44.44%) and it was followed by the genotype ARR/ARQ (37.5%). Presence of the genotype ARR/ARR was not reported during the study and it known to be most resistant genotype against the scrapie. These results could help to develop a national program to increase the genetic resistance of the Tunisian sheep population.
1 Introduction

Scrapie is an infectious disease and the susceptibility of this disease is associated with a specific gene i.e. PrP gene (alleles). These alleles are mainly differing in three codons of the gene viz 136, 154 and 171 codons (Tongue et al., 2004; Gama et al., 2006). Animals bearing a combination of sensitive alleles are at greater risk of developing the disease, while those carrying a combination of known alleles appear to be more resistant shelter (Goldmann et al., 1990; Hunter, 1996). So far, AA136 RR154 RR171 homozygosity (ARR / ARR) seems to provide a very high resistance against disease while homozygosity VV136 RR154 QQ171 (VRQ / VRQ) is associated with high sensitivity toward the disease (Hunter, 1997; Hunter et al., 1997). Between these two extremes situation of sensitivity and resistance, several intermediate alleles and their behavior response against scrapie remains the subject of much research.

The AA136 RR154 RR171 genotype (ARR / ARR) is far from ubiquitous in sheep populations evaluated. Several studies in Europe and United States indicated that there are significant variations in the frequency of different alleles between breeds, lines and regions. Some breeds shows more clear resistant than others (Hunter et al., 1997; Thorgeirsdottir et al., 1999; Tranulis et al., 1999; Drögemüller et al., 2001; Palhière et al., 2002; De Silva et al. 2003; Acin et al., 2004; Roels et al., 2004; Tongue et al., 2004). In recent years Various research work has been undertaken in various countries for finding out the information regarding the resistance and susceptible gene and these studies help researchers in making a a clear picture regarding the various resistant breed (EFSA, 2014). Different countries have already implemented various programs to control and eradicate scrapie based on the use of genetically breeding disease resistance. However, till now no program has been undertaken for the control and eradication of scrapie from Tunisia. In this context present work is aimed to characterized Tunisian sheep population on genetic resistance to scrapie.

2 Material and methods

Seven hundred and twenty blood samples (360 Barbarine “B” and 360 Western thin tail “W”) have been randomly collected from Studied area. Among these selected animals 50 percent were male while rest 50 percent were female. Blood sample from jugular vein of the both sexes of pure B and W breeds were collected in ethylenediaminetetraacetic acid (EDTA) containing vacutainer tubes and stored 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) with some modifications (Hentati et al., 2012). 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 products were purified by using PCR Purification Kit (Jena Bioscience) and eluted in 30 μl sterile ddwater, controlled in a 1.2% agarose gel containing ethidium bromide in Tris-borate EDTA buffer and visualized under UV trans illmination. Sequencing of the PCR products was carried out in an authorized laboratory with the help of 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). Genotypic frequencies were calculated from the direct counting of genotypes as follows:$f_{ij} = \frac{n_{ij}}{N}$. Where, $n_{ij}$ is the number of animals with the genotype of $ij$ and $N$ is the number of total animals.

3 Results and discussion

DNA from 720 animals was sequenced in order to establish each animal’s genotype for codons 136, 154 and 171 of the PrP gene (figure 1). All analyzed animals were A/A homoyzogus for the 136 codon. Alanin at 136 was reported to be related to resistance to scrapie (Elsen et al., 1999; Wang et al., 2008). Only a single allele H has been observed at codon 154. The importance of amino acid residue at this codon remains controversial. Indeed the presence of the H allele at codon 154 represented protection against scrapie in some breeds (Hunter, 1997), but it was susceptible in some other breeds (Acitius et al., 2006). At codon 171three different amino acid residues Q, R and H with frequencies of 72.92, 18.75 and 8.33% respectively were reported. During the study four genotypes combination viz ARQ/ARQ, ARR/ARQ, ARH/ARQ, AHQ/ARQ were observed. The results of genotyping analyzes are presented in figure 2 and table 1. Results of the study revealed that the genotypes associated with greater resistance are present in both the studied breeds.
However, the presence of the highly resistant genotype (ARR / ARR) was not observed in studied animals. Moreover, the presence of the highly sensitive allele VRQ was also not reported from all studied animals.

The knowledge gained in recent years can link concretely susceptibility to scrapie and PrP gene polymorphism. They show the greatest resistance of ARR / ARR sheep and the high sensitivity in animals VRQ / VRQ and ARQ / VRQ infected flocks. From these findings a suitable selection is possible which helps in the selection of a population resistance against scrapie (Tongue et al., 2004). Some cases of ARR / ARR sheep with scrapie have been described in recent years (Buschmann, 2004). Particularly atypical case epidemiology, injury, clinical disease and immunodiagnostic profiles were different from what is usually met for classical scrapie.
Norwegian researchers reported several atypical cases in animals other than those that are more common with classical scrapie (Moum et al., 2005) genotypes. These recent data, without questioning the whole basis of genetic selection for scrapie suggest caution in recommendations of present study. A conclusion of the study have placed emphasis on a recommendation from basic disease control: "Maintaining a closed herd severely limits the introduction of new diseases". For scrapie, this principle is particularly true with respect to the introduction of females, males are considered very low (even zero) transmission agents.

**References**


