

Prion protein gene polymorphism in four West African sheep populations

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Abstract A total of 162 individuals, belonging to three Burkinabé and one Niger sheep populations, were analysed for prion protein (PrP) gene polymorphism at codons 136, 154 and 171. The ARQ allele was the most frequent in both the Burkinabé (86.7%) and the Niger (67.5%) sheep populations. The highly sensitive allele VRQ was not found in the sampled individuals. The highly resistant ARR allele was in very low frequency in the Burkina-Sahel (4.4%) and Mossi (3.2%) populations and was not present in the Djallonké and Touareg populations. Only 4 out of 15 possible PrP genotypes were identified in the sampled individuals. No favourable ARR/ARR genotypes were found in either of the breeds. Sequencing a subgroup of the samples allowed the identification of other five polymorphisms on the PrP gene sequence at codons 116, 138, 151, 237 and 240. The very low frequency of the ARR allele in the West African sheep should dissuade the implementation of a preventive selection programme aimed to increase resistance to scrapie, to avoid an extreme erosion of the genetic stock.

Keywords PrP · Scrapie · RT-PCR genotyping · Sahelian sheep · Dwarf sheep · West Africa

Introduction

Scrapie is one of the diseases of the group of transmissible spongiform encephalopathies, which include Creutzfeldt–Jakob disease in humans and bovine spongiform encephalopathy (BSE) in cattle. The variations at amino acid codons 136 (A/V), 154 (R/H) and 171 (Q/R/H/K) of the PrP gene have been shown to be particularly important in genetic susceptibility to classical scrapie (Heaton et al. 2003; Lühken et al. 2008). Polymorphisms at other codons are rare and have not yet been associated with classical scrapie susceptibility. Nowadays, there is an increasing interest to know PrP genotype and allelic frequencies in different sheep populations to carry out association studies with clinical scrapie (Ekateriniadou et al. 2007; Lühken et al. 2008), ascertain how selection for scrapie resistance can affect selective programmes for performance traits (Alfonso et al. 2006) or conservation programmes (Álvarez et al. 2007, 2009a; Windig et al. 2007), and document the variability of PrP gene in areas in which, to date, scrapie cases are few or have not been reported (Gootwine et al. 2008; Passos et al. 2008; Ün et al. 2008) to prepare for possible classical scrapie outbreaks (Kipanyula et al. 2009; Álvarez et al. 2011).

Although many studies have described the genetic susceptibility of scrapie in European sheep breeds, little is known about sheep populations with other origins. Reports on PrP polymorphism in African sheep are scant (Serrano et al. 2007; Kipanyula et al. 2008, 2009). There is an increasing interest in the assessment of African sheep diversity (Nsoso et al. 2004; Almeida 2011). The aim of this research is to ascertain the genotypic and allelic frequencies of PrP

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gene in three Burkina Faso and one Niger sheep populations (Traoré et al. 2008a) to increase the information on this issue in Africa and gain understanding on the genetic diversity in West African sheep (Álvarez et al. 2009b).

Materials and methods

Blood samples were obtained from a total of 162 reproductive-age individuals belonging to the three Burkinabé sheep populations: (a) Burkina-Sahel (46), kept in the arid Sahel area covering the Northern part of Burkina Faso; (b) Djallonké (50), kept in the Sudan area, covering the Southern part of Burkina Faso; (c) Mossi (46), located in the Sudan-Sahel area, covering the central part of the country and one Niger sheep population: (d) Touareg (20). Further description of sampling and environmental conditions in which the Burkinabé sheep breeds are managed can be found in Traoré et al. (2008a) and Álvarez et al. (2009b).

Total DNA was isolated from blood samples using the Invisorb® Spin Micro DNA Kit (Invitek, Berlin, Germany) according to the manufacturer instructions. Diagnostic of PrP polymorphisms at codons 136 (A/V), 154 (R/H) and 171 (R/H/Q/K), were carried out on all individuals using a dual fluorescent multiprobe assay consisting of two closed tube PCR reactions containing four dual-labelled fluorescent ASO probes each, for the detection in real-time of the allelic variants of sheep PrP gene mentioned above. This protocol was run in an iCycler (Biorad, Barcelona, Spain), and is coincident with that described by Van Poucke et al. (2005) as modified by Traoré et al. (2008b) with the addition of a new fourth probe for the 171K polymorphism in the second PCR reaction (see Table 1 at this reference and 171K: Cy5-AGACCAGTGGATAAGTATAGTAACCA- BHQ2).

A fragment of 428 bp of the PrP gene, including amino acids from 99 to 240, was amplified on five Burkinabe animals for which the standard genotyping assay failed using primers: for: 5'-GTGGAGGCTGGGGTCAAGGTGGTAG-3' and rev: 5'-AAAGAGATGAGGAGGATCACAGGAC-3'. PCR conditions were as follows: the final reaction volume was 10 µl; the reaction mixture consisted on 50–100 ng of genomic DNA, 0.2 U of Taq polymerase (Biotools, Madrid, Spain), 0.25 µM of each primer (Sigma-Aldrich, Madrid, Spain), 200 µM of each dNTP (Biotools, Madrid, Spain) and 2 mM MgCl₂. The PCR protocol were run in an GeneAmp® PCR System 9700 (Applied Biosystems, Madrid, Spain) and included an initial step of 95°C (3 min), followed by 40 cycles of 30 s at 95°C for DNA denaturation, 30 s for primer annealing at 55°C and 30 s at 72°C for primer extension. PCR product was purified with the exoSAP-IT® protocol (USB, Barcelona, Spain) and sequenced in both strands by using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Madrid, Spain) following manufactured recommendations, on a ABI310 sequencer analyser (Applied Biosystems, Madrid, Spain). Sequences were appraised by eye and aligned by using Clustal in the MEGA 4.0 software (Tamura et al. 2007).

For informative purposes, individual genotypes were classified in five risk groups from R1 (very low) to R5 (greatest risk), according to the European Union regulations (Álvarez et al. 2007). A brief description of the characteristics of the risk groups is as follows: R1, very low risk both at the individual and at the progeny level; R2, low risk both at the individual and at the progeny level; R3, low risk at the individual level and not low at the progeny level depending on the genotype of the other parent; R4, scrapie occasionally recorded at the individual level and higher risk than in R3 progeny at the progeny level; and R5, greatest risk both at the individual and at the progeny level

Table 1 Genotypic and allelic frequencies for the PrP gene in four West African sheep populations. Frequencies are given in absolute values and as percentages (in brackets). Classification of PrP genotype in risk groups and sample size per population are also given

	Risk group	Population				Total
		Burkina-Sahel	Djallonké	Mossi	Touareg	
Sample size		46	50	46	20	162
Allele						
AHQ		6 (6.5)	8 (8.0)	9 (9.8)	13 (32.5)	36 (11.1)
ARQ		82 (89.1)	92 (92.0)	80 (87.0)	27 (67.5)	281 (86.7)
ARR	Highly resistant	4 (4.4)	–	3 (3.2)	–	7 (2.2)
Genotype						
AHQ/AHQ	R2	–	1 (2.0)	1 (2.2)	1 (5.0)	3 (1.9)
ARR/AHQ		1 (2.2)	–	–	–	1 (0.6)
ARQ/AHQ	R3	5 (10.9)	6 (12.0)	7 (15.2)	11 (55.0)	29 (17.9)
ARR/ARQ		3 (6.5)	–	3 (6.5)	–	6 (3.7)
ARQ/ARQ	R4	37 (80.4)	43 (86.0)	35 (76.1)	8 (40.0)	123 (75.9)

Genotypic (f_{ij}) and allelic (p_i) frequencies were calculated, respectively, as $f_{ij} = \frac{n_{ij}}{N}$ and $p_i = \frac{2f_{ii} + \sum f_{ij}}{2}$, where n_{ij} is the number of animals with the genotype ij and N is the number of total animals studied in each breed (Gama et al. 2006).

Results

Genotypic and allelic frequencies of the PrP gene for the sampled populations are given in Table 1. The ARQ allele was the most frequent in both the Burkinabé (86.7%) breeds and Niger (67.5%) breeds. The highly sensitive allele VRQ was not found in any of the sampled individuals. The highly resistant ARR allele was at very low frequency in the Burkina-Sahel and Mossi populations and was not present in the Djallonké and Touareg populations. Five out of 15 possible PrP genotypes were identified in the sampled individuals. The ARQ/ARQ genotype, which is classified at the undesirable risk level R4, was the most frequent in the all the African sample populations (75.9%). No favourable ARR/ARR genotypes were found.

Five Burkinabé samples did not give consistent results at codons 136 (A/V) and 171 (R/H/Q/K). These samples were sequenced to allow a correct diagnostic. No new polymorphism was found on codons 136, 154 or 171. However, seven mutations at codons 116, 138, 143, 151, 172, 176 and 240, as well as two silent mutations at codons 231, 237 were found (Table 2).

Discussion

Among breeds, there is a large variation of allelic and genotypic frequencies on the PrP gene (Álvarez et al.

2007, 2009a; Gootwine et al. 2008; Lühken et al. 2008; Passos et al. 2008). In this work, the ARR allele was only detected in the Burkina-Sahel and the Mossi populations. With support in both morphological and microsatellite information, it has been suggested that the Mossi breed can be considered as a transition population formed by a continuous gene flow from Sahelian sheep into the Djallonké (West African Dwarf) breed (Traoré et al. 2008a; Álvarez et al. 2009b).

The results obtained here for the four West African sheep are consistent with the recent reports by Kipanyula et al. (2008, 2009) in Tanzanian sheep. The alleles found in these West African population were basically the same as those (AHQ, ARH, ARQ and ARR) found in these two Sub-Saharan Africa populations, with the ARQ allele frequency varying from 0.772 to 0.905 in the two studied Tanzanian sheep breeds (Black Head Persian and Red Maasai, respectively). Also, the ARQ/ARQ genotype was the most frequent in the two Tanzanian breeds (63.3% and 81.0%, respectively) and there was no presence of the ARR/ARR genotype. The ARQ allele is thought to represent the ancestral form of the PrP gene. This allele is usually present at high frequencies in breeds traditionally raised under harsh conditions (Gama et al. 2006). The overall scenario showed here may be different for Mediterranean African sheep. Serrano et al. (2007) in Moroccan sheep breeds reported that the ARR allele frequency varied from 25.85% to 31.83%; 31.83%; with the frequency of the ARR/ARR genotype being relatively high (from 4.49% to 16.28%).

The performance of RT-PCR diagnostic protocol used here has been shown to be affected when polymorphisms involve the flanking regions of the codons subject to diagnostic (Traoré et al. 2008b). The sequencing of several samples, has allowed us to demonstrate that West African sheep populations still showed some diversity on PrP gene (see Table 2), that may be profitable for future breeding programmes.

As in many developing countries worldwide, there is not a scrapie surveillance system in West Africa. Therefore, the incidence of the disease in the sampled populations, if existent, is not known. In such scenario, the implementation of a preventive selection programme aimed at increased resistance could conceivably be suggested. However, as suggested in other African sheep (Kipanyula et al. 2009), before embarking on such a scheme, it would be advisable to determine whether or not scrapie poses a problem. In any case, the results reported here should dissuade from this idea. The very low frequency of the ARR allele in West African sheep would limit the available genetic variability for breeding, resulting in extreme erosion of the genetic stock, if selection was solely for this allele.

Table 2 Additional polymorphisms on PrP gene in three native Burkina Faso sheep populations

Mutation	Population	Reference
A116P	Djallonké	This work
S138S	Mossi	This work
H143R	Burkina-Sahel, Djallonké, Mossi,	DeSilva et al. 2003; Heaton et al. 2003; Álvarez et al. 2011
R151G	Djallonké	Acín et al. 2004
Y172D	Djallonké	Acín et al. 2004; Serrano et al. 2007; Ūn et al. 2008; Álvarez et al. 2011
N176K	Mossi	Vaccari et al. 2001
R231R	Djallonké	Ūn et al. 2008
L237L	Djallonké, Mossi	This work
S240P	Mossi	This work

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