Analysis of differences in prion protein gene \((PRNP)\) polymorphisms between Algerian and Southern Italy's goats

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ABSTRACT

Goats are adapted to live in harsh areas of the world ensuring minimum levels of milk and meat production without much economic input. Scrapie is a fatal prion disease of small ruminants detected in different countries worldwide. Polymorphisms in the prion protein gene \((PRNP)\) modify the degree of susceptibility/resistance to scrapie. \(PRNP\) polymorphisms were analysed in the four main Algerian goat breeds (Naine de Kabylie, Arbia, Mozabite, Mekatia), and in two native breeds of South Italy (Aspromontana from Calabria and Cilentana from Campania). The results were compared also with two previously described Sicilian native breeds (Girgentana and Rossa Mediterranea). Seven amino acid substitutions were detected in Naine de Kabylie goat which represents the original native Berber breed. All other breeds presented no more than six variants and common polymorphisms were present at codons, 154 and 240. Isoleucine at 137 was present in Algerian breeds only. The Italian Cilentana shared more variants with Algerian breeds whereas the Aspromontana breed was the only one to show a Serine at 127. Exclusive new variants were not detected since all polymorphisms were already described in other goats worldwide. The scrapie protective allele encoding lysine (K) at codon 222 was detected in the Naine de Kabylie and M’zabite breeds at low-frequency whereas is present with frequencies higher than 10% in all the Italian breeds reared in regions with elevated scrapie incidence. The overall results showed a substantial number of polymorphisms in \(PRNP\), particularly in Naine de Kabylie breed, which carried also unique genotypes.

Introduction

Scrapie is a fatal, neurodegenerative disease in small ruminants, belonging to the group of transmissible spongiform encephalopathies (TSEs). Polymorphisms of the gene encoding the PrP protein \((PRNP)\) at codons 136 (A or V), 154 (R or H), and 171 (R, Q or H) strongly modulate susceptibility to disease in sheep (Elsen et al. 1999). In goat \(PRNP\), at least 41 amino acid substitutions and 19 silent mutations have been described in several countries (Vaccari et al. 2009), but only few of them have been associated to the outcome of the disease (EFSA 2017). Case/control studies and experimental challenges in goats demonstrated a high-protective effect of the K222 variant (Acutis et al. 2006, 2012; Aguilar-Calvo et al. 2014; Barillet et al. 2009; Corbière et al. 2013; Lacroux et al. 2014; Vaccari et al. 2006) whereas N146S/D have been associated with scrapie resistance in Cypriot goats (Papassavva-Stylianou et al. 2007). As for all African countries, scrapie has never been reported in Algerian goats but active surveillance plan for the disease has never been in place in the country. The aim of this work was the analysis of \(PRNP\) polymorphisms in the main goat breeds of Algeria in comparison with two native breeds of South Italy: Cilentana and Aspromontana and two already described Sicilian goat breeds coming from an area where scrapie cases have been detected by active and passive surveillance (Vitale et al. 2016). Cilentana goats is a breed reared mainly in the province of Salerno, especially in the Cilento and Vallo di...
Aspromontana goat is an indigenous breed reared in Reggio Calabria province in the massif of Aspromonte in a mountainous harsh region. Both breeds are reared for milk and meat. All Italian breeds are described in www.agraria.org web site. The total number of goats in Algeria exceeds 5.129 million heads (FAOStat2014) characterised by their great diversity and heterogeneity due to uncontrolled mixing of local populations with imported breeds. Algerian goats are hardy and adaptable to rear in difficult areas representing a significant source of milk and meat for human consumption (Fantazi and Bouhadad 2005). The traditional breeding of goats is economically relevant, especially in Saharan populations. Four main native breeds are present in Algeria each one mainly located in different areas of the country: Arbia (the largest population) is in steppic regions, Naine de Kabylie in mountainous regions of Kabylie, Mekatia in the area between the steppic and Saharan region, and M’zabite throughout the northern part of Sahara. Recent analysis on the phenotypic variability (Ouchene-Khelifi et al. 2015) among Algerian breeds show distinct aspects such as long drooping ears and absence of topknots for Arbia breed only. Photos of the four breeds are shown in Figure 1 (A,B).

Materials and methods

Sampling and studied area

Blood samples were collected from 200 goats, belonging to 31 herds of four of the main Algerian native goat breeds. The sample consisted of 75% males plus 25% of females with no genetic relation. The animals belonged to the Naine de Kabylie (n = 56) sampled in the coastline and first highlands; Arbia (n = 68) from the steppe and part of the Sahara (region of Tiaret, Djelfa, Biskra); Mekatia (n = 45) specifically located in the region of Laghouat; M’zabite (n = 31) from the region of Ghardaia (Figure 2). Blood samples of the South Italy breeds were collected from reproductive males only. Aspromontana (61 animals from 9 farms) in Calabria and Cilentana (49 animals from 18 farms) in Campania.

Genetic analysis

Polymerase chain reaction (PCR) amplification of the open reading frame of the caprine PRNP and direct PCR fragment sequencing was carried out as previously described (Migliore et al. 2015). Hardy-Weinberg equilibrium (HWE) was calculated with Genotype Transposer (Version 1.0) (Cox and Canzian 2001).
To determine the allelic frequencies, we counted the number of allelic variant in each single \(PRNP\) locus and divided by the total number of alleles across the breed \(p = i/(nN)\). The genotypes combination at frequencies \(>3.5\%\) are described. We put this cut-off to exclude genotype combinations relative to polymorphism detected in single animals.

**Results**

The coding region of the goat \(PRNP\) was sequenced in a total of 197 Algerian native goat breeds and a total of 110 Cilentana and Aspromontana. Silent mutations were present at codons 42, 138 and 179 but aminoacid substitutions were observed in nine codons: T110P, M137I, R139S, I142M, H143R, R154H, R211Q, Q222K and S240P. \(PRNP\) haplotypes derived from known mutations were deduced on the basis of previous reports (Vaccari et al. 2009). The polymorphisms 154 and 240 were present in all breeds; isoleucine (I) at codon 137 in Algerian breeds only with a moderate frequency (3–7%). Histidine (H) at codon 154 (H154) showed a frequency of 16–26% in Algerian breeds and 13% in Cilentana. In Aspromontana, lower frequencies were detected (6.3%) similar to the previously analysed Sicilian goats Girgentana and Rossa Mediterranea and a unique variant with a serine at codon 127 was also observed (Table 1). The main \(PRNP\) variants represented by serine or proline at codon 240 were previously observed in European and other North African goats (Kdidi et al. 2014; Serrano et al. 2009; Vaccari et al. 2009 ). The polymorphism M142 was shared among Naine de Kabylie, Mekatia, M’zabite and Cilentana with a frequency from 1.6 to 2.3% (Table 1). The S139 variation showed a frequency of almost 5% in M’zabite and Naine de Kabylie but was absent in Italian and in the other two Algerian breeds. Glutamine at codon 211 (Q211) was found in Arbia, Naine de Kabylie and Cilentana. Arginine (R) at codon 143 was detected with a low-frequency in Mekatia breed only (2.3%) whereas an individual Arbia goat was heterozygous for proline (P) at codon 110. The same polymorphism was detected in Cilentana goats with a frequency of 2.3%. The lysine (K) at codon 222, which is related with scrapie resistance in European goats was described in Naine de Kabylie at 0.9% and in M’zabite at 6.5% frequency. In contrast, higher frequencies (>10%) are present in all breeds of South Italy. Italian goats and Arbia populations were in Hardy–Weinberg equilibrium (HWE, \(p > .05\)). The populations of Mekatia, M’zabite and Naine de Kabylie were not consistent with HWE (\(p < .05\)). The distribution of the most frequent genotypes in Algerian goats (frequency \(>3.5\%\)) showed that Arbia was the only breed carrying the genotype I137M, whereas M’zabite was the only one for the resistant Q222K. H154H and Q221R were present only in Naine de Kabylie goats (Table 2).

**Discussion**

The results reveal a large number of polymorphisms with seven aminoacid substitutions in \(PRNP\) gene (Table 1) in Naine de Kabylie that give rise to a large combination of genotypes (Table 2). More \(PRNP\) variability was observed among Algerian goats compared to the South Italian breeds that shared mainly five variants among them (R143, H154H, Q168, P240 and K222). All polymorphisms have been reported in other goat breeds worldwide (Vaccari et al. 2009) at different frequencies and some of them are related to a major resistance or susceptibility to scrapie in goats. The two...
most frequent variants S240P are typical of goats and P240 may be associated with a partial protection from clinical TSE but not from prion infection (Bouzalas et al. 2010). All native Algerian goats and Italian Cilentana showed the polymorphism H154 at frequency above 13% as in Tunisian (Kdidi et al. 2014) and in Chinese goats (Zhang et al. 2004). The polymorphism H154 has a variable protective effect against TSE probably depending on the genetic background of breeds (Billinis et al. 2002). However, the homozygous genotype H154 was present only in Naine de Kabylie (frequency 5.45%, Table 2 in bold). A lower frequency of this polymorphism was found in the other Italian breeds (Table 1) as in Chaouni and D’man goats in Morocco (Serrano et al. 2009) and in goats of Spain (Acín et al. 2013) and Greece (Kanata et al. 2014). The isoleucine at codon 137 (I137) was found with a frequency ranging from 3.2 to 7.5% in all native breeds of Algeria as well as in Chaouni and D’man goats in Morocco (2.5–4%) (Serrano et al. 2009) and in Tunisian goats from 1 to 3% (Kdidi et al. 2014). It was completely absent in the Italian breeds in this study but it has been reported in other South Italian goats (Acitis et al. 2008) and in Spain 0.5% (Acín et al. 2013). The serine at codon 139, found in M’zabite and Naine de Kabylie at almost 4.5% frequency, have been already reported in Moroccan D’man goat (Serrano et al. 2009) and in Tunisian goats (Kdidi et al. 2014), whereas in Europe, it was only shown in Moncaina goat in Spain at very low-frequency; however, its implication in the susceptibility to scrapie disease could not be evaluated due to the low number of the analysed animals (Acín et al. 2013). The polymorphism M142, was already described in European and in Moroccan goats as commonly linked to P240 (Vaccari et al. 2009; Serrano et al. 2009). In this study, it was present in the Italian Cilentana at similar frequency of Algerian goats and linked to P240; however, in one Mekatia goat, it was unusually associated with S240. The variant S/D146, which is high prevalent in Cyprus goats conferring resistance to the disease (Papasavvastylianou et al. 2007) was not found in Algerian and Italian breeds as in the other North-African and European goats. However, this polymorphism is also present with a minor frequency in Greece (Kanata et al. 2014) and Turkey (Meydan et al. 2017) suggesting that this resistance-associated polymorphism may be maintained in East-European breeds related to Damascus goat independently of the presence of scrapie (Meydan et al. 2017).
A polymorphism with a proline at position 110 (P110) present in many European breeds, was detected in a single Arbia goat and in Cilentana at 2.3% but it is absent in all other breeds. The two breeds are both related to Damascus goats (Syrian goats). Arginine (R) at codon 143, described already in a single Tunisian goat, was detected in this study in Aspromontana (6.3%), Cilentana (13.2%) and is also present in the Sicilian breeds. Among Algerian goats, it was detected in Mekatia breed only (2.3%). The glutamine at codon 211 (Q211), previously described in North Italy (Acutis et al. 2008), in Spain (Acín et al. 2013), in Greece (Kanata et al. 2014) and Tunisian goats (Kdidi et al. 2014), was detected in a single Arbia goat (0.7%), but it showed a moderate allelic frequency in Naine de Kabylie (3.6%) and in Cilentana (3.4%) (Table 1) combined as heterozygous genotype R211Q at 3.6% (Table 2). The 211 (R/Q) polymorphism as well as others at codons 142 (I/M), 154 (R/H) and 222 (Q/K), were previously found to induce a significant degree of protection towards natural scrapie infection in French Alpine and Saanen breeds (Barillet et al. 2009). However, a recent study showed its strong relation to scrapie resistance in Canadian dairy goats independently from K222 presence (Srithayakumar et al. 2016). The South Italian breeds showed six polymorphisms and both confirmed a high-level of the allele resistant K222 as previously observed for the Sicilian goats (Migliore et al. 2015; Vitale et al. 2016) where a relation with high-scrapie incidence has been postulated (Migliore et al. 2017). Scrapie incidence is higher in South and Central Italy compared to the northern regions and the highest incidence has been observed in some provinces of Sicily, Sardinia and Central Italy probably related to the usage of a contaminated vaccine as shown in Figure 3 (Bertolini et al. 2012). High-frequencies were detected in Aspromontana (10.3%) and Cilentana (18.2%) similar to the previously described Sicilian goats (Girgentana and Rossa Mediterranea in Table 1) in which a direct relation of its high prevalence with the high-scrapie incidence has been postulated (Migliore et al. 2017). In Italy, northern breeds showed low-frequencies of K222 (Acutis et al. 2008) but also a lower incidence of scrapie as reported in Bertolini et al. (2012). As shown in Figure 3, Aspromontana and Cilentana are both reared in provinces with an incidence of the disease between 0.2 and 0.84. In Puglia, another region of South Italy, an ARR frequency up to 50% has been detected in sheep (Martemucci et al. 2015). In central regions, studies on goat and sheep have reported a frequency of 7% for K222 in Umbria and Marche (Biagetti et al. 2016) and 32% of the resistant allele ARR has been detected in a native sheep breed (Curcio et al. 2015).

The scrapie resistant variant K222 in Algerian breeds was present in Naine de Kabylie at very low-frequency as in Tunisia and Morocco, whereas a 6.5% frequency was detected in M’zabite (Table 1) giving rise to a good frequency of genotypes also (Table 2) Similar frequencies have been detected in goats in Greece (Kanata et al. 2014) and in Southern Italy (Acutis et al. 2008) where scrapie outbreaks have been recorded. The analysis on all breeds showed that Naine de Kabylie carried the highest number of PRNP polymorphisms and genotypes combinations (Tables 1 and 2), which might be consistent with its origin. This goat breed described as the “Berber goat”, has its ancient origin in Neolithic period and it has been considered the original North African goat present before the migration of goats from the Asia Minor. It is characterised by a small size, long hair, drooping ears and its skeleton has preserved the main features of the fossil goats of the Neolithic. It has been preserved in all the mountain areas of the littoral, from Kabylie to Morocco and is ubiquitous throughout the Maghreb.
(Esperandieu and Chaker 1994). Its ancient origin and uncontrolled genetic mixing with other native and foreign breeds can explain this high polymorphism in PRNP and that this oldest original Berber goat has undergone major changes compared to the Arbia breed, which resulted in a more stable among the four in recent morph-biometric analysis of the four breeds (data not shown). In this paper, Arbia and Mekatia showed only five polymorphisms in contrast to the seven of Naine de Kabylie and the six of other breeds. The South Italian breeds have in common high-level of the resistant allele K222 probably related to the scrapie incidence. In Algeria, no surveillance for the disease have been put in place but scrapie in small ruminants as all other TSEs are rare diseases with a late onset in nature. TSE originated as health emergencies in developed countries essentially for risk factors related to human activities. In Italy, a relation of high-scrapie incidence in small ruminant with the administration of a contaminated vaccine has been already suggested (Bertolini et al. 2012) and this can be also related to the high prevalence of protective alleles in some areas in both sheep and goat populations (Vitale et al. 2016).

The genetic variability of Algerian goat populations have been poorly studied and unfortunately external goat breeds, such as Saanen, were introduced in Algeria since 1970 to increase milk production (Kadi et al. 2013). The uncontrolled mixing among local breeds in addition to imported breeds (Maltese, Saanen and Alpine) in the seventies is probably the cause of the detection of several overlapping features among the native and foreign breeds. In PRNP, for example, some polymorphisms such as 142M and 211Q have lower frequencies (1.6–2.3% and 0.7–3.6% respectively) in Algerian goats compared to an average of 6.1–7.2% for 142M and 10.2–12.2% for 211Q Saanen in Alpine goats from France (Barillet et al 2009) and Italy (Acutis et al 2008). However, these two polymorphisms were not present in pure Mediterranean goats in Sicily (Vitale et al. 2016).

Algeria represented an important stage in goat colonisation from Southwest Asia into North Africa between 7,000 BP (before present) and 6,000 BP (Hassan 2000) Subsequently, goats were spread rapidly from the Middle East mainly to the Central Sahara and the Ethiopian highlands between 6,500 BP and 5,000 BP (Clutton-Brock 2000; Newmann 1995). The subsequent dispersal of domestic goats in Northern Africa was influenced by overland and maritime diffusion through the Sahara desert and Mediterranean Sea respectively, especially in the coastal regions of pastoralist societies whose economy included goat herding (Pereira et al. 2009). The maintenance at the maximum level of performance of goats perfectly adapted to harsh geographic conditions can be important to preserve biodiversity of goat in Algeria hampering what happened with sheep population in which more detailed genetic studies showed the potential threatens for its biodiversity (Gaouar et al 2012, 2015).

Conclusions

Several polymorphisms in PRNP have been shown in our analysis, particularly in the more ancient African Naine de Kabylie, the oldest breed in North Africa. The resistant allele K222 was present at high-level in South Italian breeds, at very low-frequency in Naine de Kabylie whereas frequency similar to other Mediterranean countries were detected in M’zabite. The overall results showed how polymorphisms in PRNP of goat populations from different areas of Mediterranean basin can differ in terms of variability and relative frequencies.

Ethical approval

Blood samples from the animals were collected under routinary work upon breeder’s approval. No experiment were performed on the animals.

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Disclosure statement

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