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Short communication

Molecular and genealogical analyses reveal multiple sources of the mutation associated with dermatosparaxis in Brazilian White Dorper sheep

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ABSTRACT

Dermatosparaxis, also known as Ehlers-Danlos syndrome, is a recessive genetic disorder characterized by defects in collagen production, leading to skin fragility and delayed wound healing. In sheep this disease is associated to a transversion c.421G > T located on exon 2 of the gene ADAMTS2 and presents a special importance for the White Dorper breed. This study aims to identify carriers for the mutation associated with dermatosparaxis in Brazilian White Dorper herds and also to better understand the origin and the possibility of dissemination of this disease combining molecular and genealogical approaches. Genotyping by PCR-RFLP revealed the presence of seven out 66 heterozygotes, resulting in an estimated frequency of 5.3% for the dermatosparaxis associated allele in Brazilian White Dorper sheep. Pedigree analysis permitted the identification of two separate groups of individuals carrying the dermatosparaxis associated allele, one represented by descendants from individuals imported from South Africa and the second originated from animals imported from Australia. These findings suggest that the implementation of a program aiming to eradicate/control of dermatosparaxis in Brazilian White Dorper sheep is needed and the success of this strategy initially depends on the utilization of a molecular testing to detect carriers.

1. Introduction

The advent of breeding programs together with the use of reproductive bio techniques tends to optimize the genetic progress in livestock. However, the use of a small number of individuals with high genetic merit can contribute to the increase of inbreeding levels, leading to an increase in the probability of dissemination of recessive genetic diseases. The implementation of programs aiming to eradicate/control these diseases must occur even if the deleterious alleles are in low frequencies, because once the frequency of these alleles is high in a population, its eradication tends to be more expensive and difficult (Leroy and Baumung, 2010). The use of molecular methods allows the identification of heterozygotes and therefore facilitates the eradication/control of alleles associated with recessive genetic diseases (Shuster et al., 1992).

Studies related to genetic diseases in livestock are incipient in Brazil, principally involving small ruminants. The majority of these studies are restricted to a description of clinical and pathological findings and there are only a small number of studies using molecular techniques to describe the mutations associated with these diseases (Passos et al., 2009; Andrade et al., 2014; Dantas et al., 2014; Silva et al., 2015). Besides, studies involving a combination of molecular and pedigree analyses aiming to better understand the origin and the risk of dissemination of these diseases do not exist in Brazil.

Among the genetic diseases identified in Brazil, the dermatosparaxis (also known as Ehlers-Danlos syndrome) can be highlighted. This disease is characterized by defects in collagen production and it was described in several livestock species (The Merck Veterinary Manual). The clinical signs include fragility in skin and delayed wound healing. In sheep this condition can be associated with rupture of the gastrointestinal tract and arterial aneurisms and may often be fatal (The Merck Veterinary Manual; Vaatstra et al., 2011; Zhou et al., 2012; Andrade et al., 2014; Silva et al., 2015). The

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transversion c.421G > T located on exon 2 of the gene ADAMTS2 was identified as the causal mutation in White Dorper sheep breed and it is presumed that it creates a premature stop codon resulting in a truncated ADAMTS2 protein that contains only the signal peptide and part of the pro-domain (Zhou et al., 2012).

Countries such as Australia, New Zealand and United Stated have implemented programs based on molecular diagnostic to detect and eliminate dermatosparaxis associated allele carriers from White Dorper herds (Vaatstra et al., 2011; Andrade et al., 2014). However, in Brazil there is no program aiming to eradicate/control this disease and recently two studies described the presence of affected individuals in Brazilian White Dorper population (Andrade et al., 2014; Silva et al., 2015).

Thus, the aims of this study were the identification of carriers for the mutation associated to the dermatosparaxis in Brazilian White Dorper herds and better understand the origin and the possibility of dissemination of this disease through the combination of molecular and genealogical approaches.

2. Material and methods

2.1. Sample collection

Sixty seven blood samples from White Dorper sheep individuals belonging to five flocks located in Bahia and Sergipe states (Brazil) were collected through venipuncture using vacuum collection tubes with K_2 EDTA.

2.2. DNA extraction

DNA extraction was performed using a salting out procedure proposed by Regitano et al. (2007). Verification of quality and quantity of DNA was performed using 1,5% agarose gel electrophoresis and then the DNA was diluted to a final concentration of 40 ng/μL.

2.3. Polymerase chain reaction (PCR)

Partial amplification of the ADAMTS2 gene was performed using the primers DSX1 (5′-CCCTTCCCTTCGCAGTGG-3′) and DSX2 (5′-GAGACAAGCGCGTACTCAC −3′), described by Zhou et al. (2012). PCR conditions were: 0.4 mM dNTP; 0.4 μM each primer; 1 mM MgCl2; 2U Taq DNA polymerase; 1X buffer and 120 ng DNA, in a final reaction volume of 30 μL. The amplification steps were: (1) 95 °C, 5 min; (2) 95 °C, 1 min; (3) 64 °C, 1 min; (4) 72 °C, 1 min 30 s; (5) Repeat steps 2–4 35X; 6) 72 °C, 10 min.

2.4. Digestion of PCR products using BSTXI restriction enzyme

The amplicons were digested using *BstXI* restriction enzyme (New England Biolabs®), according to the following conditions: 10U *BstXI*; 1X digestion buffer and 10 μL PCR product. Samples were incubated at 37 °C for 3 h, followed by a step of enzyme inactivation at 80 °C for 20 min.

2.5. Genotyping

To determine the genotype of the individuals, the digestion products were submitted to a 2% agarose gel electrophoresis for 3 h and visualized using a transluminator. Homozygote individuals for the dermatosparaxis associated allele (TT genotype) present a unique fragment of 518 bp; homozygotes for the wild allele (GG genotype) present two fragments (406 bp and 112 bp) and heterozygotes (TG genotype) present three fragments (518 bp, 406 bp and 112 bp).

Table 1
Distribution of sampled animals.

Flock	Location	Sampled animals	Carriers
01	Bahia	12	2
02	Bahia	14	3
03	Sergipe	38	1
04	Bahia	2[a]	1
05	Bahia	1	0

[a] One individual of this herd was excluded from the analyses because it could not be genotyped.

2.6. Validation of the genotyping procedure

Two samples (one identified as homozygote for the wild allele and one identified as heterozygote) were sequenced (using both forward and reverse primers) using the Sanger method. The genotypes were determined after the processing of electropherograms using the Phred/Phrap/Consed suite (Ewing and Green, 1998; Gordon et al., 1998).

2.7. Pedigree analysis

The unique identification for each animal was recorded and the genealogical information was retrieved from the Brazilian Sheep Breeders Association database (http://www.arcoovinos.com.br). Pedigree visualization and analysis was performed using Endog v4.8 (Gutiérrez and Goyache, 2005) and the R packages kinship2 (Sinnwell et al., 2014) and Pedigree (Coster, 2013).

3. Results and discussion

Genotyping by PCR-RFLP revealed 59 homozygotes for the wild allele and seven heterozygotes. One individual was excluded from the subsequent analyses because it could not be genotyped. Carriers for the dermatosparaxis associated allele were distributed in four of the five flocks analyzed (Table 1).

Sequencing of the two samples (one identified as homozygote for the wild allele and one identified as heterozygote using the PCR-RFLP approach) showed a perfect match between the genotypes identified using both techniques, validating the approach used in this study as a reliable method to be applied in screening programs aiming to identify carriers for dermatosparaxis in sheep flocks.

Based on the observed results, genotype frequencies estimated assuming Hardy-Weinberg equilibrium were: 89.7% (GG), 10.0% (TG), 0.3% (TT).This implies in an estimated frequency of 5.3% for the dermatosparaxis associated allele in White Dorper sheep in Northeastern region of Brazil, which is considerably lower than the findings from Zhou et al. (2012), that identified a frequency of 10.8% for the dermatosparaxis associated allele in New Zealand flocks.

It is important to note that the number of sampled individuals in the present study is relatively small; therefore estimated allele and genotype frequencies must be evaluated carefully. However, considering the lack of studies aiming to investigate the distribution of alleles associated with genetic diseases in small ruminant populations in Brazil (Passos et al., 2009), the present study can be considered a starting point to better understand the risk of dissemination of the dermatosparaxis in Brazilian White Dorper herds, principally by the fact that dermatosparaxis associated allele was found to be present in almost all tested flocks.

The frequency for the mutated allele found in this study is similar to the results from studies aiming to describe another genetic disease, the Spider Lamb Syndrome, in Suffolk and Hampshire Down sheep breeds on which the authors suggest the use of routine molecular testing (Drögemüller et al., 2005; Passos et al., 2009). Inclusion of molecular testing in breeding programs will reduce the frequency of carriers, leading to decreased probability of birth of