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Short communication

Hereditary microphthalmia in Texel lambs in Brazil

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ABSTRACT

On a farm in southern Brazil, four lambs in a flock of 300 Texel sheep were born with bilateral blindness. They revealed bilateral occlusion of the eyelids and were unresponsive to external visual stimuli and were disoriented when walking. A post-mortem examination revealed bilateral occlusion of the eyelids and, microscopically, total or partial absence of the lens was observed. Blood samples from 20% of the herd (61/300) were sent for molecular analysis. The four affected lambs were homozygous (C/C) for a mutation (single-nucleotide polymorphism c.338G > C) in the *PITX3* gene, and 26.2% (16/61) of the herd, including the two ewe and two ram parents, were heterozygous (G/C). Based on clinical, pathological and molecular analysis, it was possible to determine the existence of hereditary microphthalmia in Texel lambs associated with the c.338G > C SNP in the PITX3 gene for the first time in Brazil. The result of this study provides a warning to veterinarians and breeders, emphasizing the importance of considering this disease in the differential diagnosis of congenital diseases in the Brazilian Texel flock.

1. Introduction

Autosomal recessive hereditary ovine microphthalmia (OMO) is a congenital disorder characterized by small eyes and total blindness in newborns as a result of ocular abnormalities in fetal development (Graw, 2003; Van der Linde-Sipman et al., 2003; Tetens et al., 2007). The condition may occur in several mammalian species, including humans. In sheep, microphthalmia predominantly occurs in the Texel breed (Tetens et al., 2007; Becker et al., 2010) as an autosomal recessive hereditary disease and is caused by a c.338G > C single-nucleotide polymorphism (SNP) in the *Paired-Like Homeodomain 3* (*PITX3*) gene. When homozygous, this polymorphism leads to abnormal development of the lens vesicle in embryonic eye development period, stage characterized by formation of the lens placode, a thickening of the surface ectoderm that comes into contact with the optic vesicle. Coordinated invagination of the lens placode and the optic vesicle results in the formation of the lens vesicle and a double-layered optic cup and provides the first indication of the final shape of the eye (Becker et al., 2010).

The disease was first described in European Texels in the 1950s (Zwiep, 1958). Since then OMO has been reported in Australia and New Zealand (Roe et al., 2003; Jolly et al., 2004; Williams, 2010).

In Brazil, there is neither any evidence of OMO reported in the scientific literature nor any research into this disease to prevent the spread in flocks. The objective of this study was to describe the clinical and pathological aspects of the OMO observed in Texel lambs and determine the existence of a genetic mutation associated with the disease in the affected lambs and their parents.

2. Materials and methods

2.1. Clinical and pathological evaluation

During a technical service visit, a producer of Texel sheep in southern Rio Grande do Sul (Brazil) notified the birth of blind sheep in the flock. After epidemiological investigation and clinical evaluation were conducted, one of the animals was euthanized and sent for pathologic evaluation. During the necropsy, fragments of all the organs were collected. Part of the material was frozen, and the remainder fixed in 10% formalin. The eyes were fixed in Davidson's solution for 24 h and sectioned in the paramedian sagittal plane. After fixation, the specimens were embedded in paraffin and processed routinely for

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hematoxylin and eosin (HE) protocol.

2.2. DNA extraction for analysis of genetic mutation

Blood samples were collected from 20% of the herd (61/300), including four affected lambs, two rams and two progenitor ewes. The other two mothers of affected lambs were previously removed from the farm. Blood was collected in tubes containing EDTA[1] and stored under refrigeration until DNA extraction. Fragments of the lymphoid organs of the necropsied lamb were also subjected to molecular analysis for the detection of Bluetongue virus and Pestivirus.

Genomic DNA was extracted from blood samples with an illustra™ blood genomicPrep Mini Spin Kit[2] according to the manufacturer's instructions. After extraction, the DNA was tested for purity and concentration in a NanoDrop® 2000 spectrophotometer.[3]

2.3. Polymerase chain reaction (PCR) and sequencing

PCR was performed using specific primers that amplify a 533-bp fragment containing the c.338G > C SNP (Becker et al., 2010) in the PITX3 gene (GenBank™ FN432136). The primer sequences were as follows: JP_PITX3_ov_Forward 5′-CTGGCACTATCTCTGGTTCTTC-3′ and JP_PITX3_ov_Reverse 5′-AATGGAGGCGGAAATGGAG-3′. The PCR was standardized to a final volume of 25 µL (12.5 µL GoTaq® Green PCR Master Mix; 500 nM of each primer; 8.0 µL nuclease-free water; 2.5 µL extracted DNA). PCR products were analyzed with 1.5% agarose gel electrophoresis. The products with the correct size were purified (GenElute™ PCR Clean-Up Kit[4]). To sequence the DNA, 10 µL of each PCR product, 5 µL of the purified forward primer and the BigDye® Terminator Cycle Sequencing Kit were used. The sequences were obtained using an ABI 3500 Genetic Analyzer and the electropherograms were analyzed using Sequencher™ 5.1[5] and compared with the normal sheep PITX3 gene sequence (GenBank™ FN432136) using BLAST (Basic Local Alignment Search Tool, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results and discussion

3.1. Clinical and pathological evaluation

In a flock of 300 Texel sheep, four lambs, approximately 20 days old (from two different mothers) were born blind. The clinical signs were characterized by bilateral blindness, no response to external visual stimulation and disorientation during walking when placed apart from the mother ewes. Information provided by the farmer indicated two Texel rams coming from a farm of the surrounding city were introduced into the flock as replacements 10 months ago. The owner couldn't designate which rams in the flock were the parents of the clinically blind animals.

One of the affected lambs was euthanized and necropsied. Gross examination revealed a 90% bilateral occlusion of the eyelid (similar to blepharophimosis) and the eyeballs were reduced in size, measuring approximately 1.8 × 1.4 cm (left) and 1.7 × 1.3 cm (right) (Fig. 1). On the cut surface, there was no distinction between the anterior and posterior chambers, with a whitish mass totally occupying the vitreous cavity and a blackish central halo (Fig. 2). The remaining organs did not show any abnormality. Microscopically, there was no delimitation of the anterior and posterior chambers, there was blackened granular pigment deposition in the cornea, and Descemet's membrane was absent. There was disorganization of the iris and ciliary body, and the lens was either absent or present as discrete fragments. The central area of the eyeball was filled by mesenchymal tissue composed of spindle cells, sometimes with vesicular epithelial formations delimited by squamous epithelium and photoreceptor cells forming rosettes, as well as the discrete and occasional proliferation of well-differentiated chondrocyte nodules and undifferentiated nerve tissue. Retinal detachment with hypertrophy of the pigment epithelium was noted at the edges of the lesion (Figs. 3 and 4).

Fig. 1. Twenty-day-old Texel lamb with microphthalmia. It presents with significant occlusion of the ocular eyelids, and the eyeball is not easily visible.

3.2. Genetic test

The c.338G > C SNP in the *PITX3* gene, responsible for microphthalmia in Texel sheep (Becker et al., 2010), presented homozygosity (C/C) in 6.5% (4/61) of the studied animals (the four affected lambs). The molecular test also indicated that 26.2% (16/61) of the herd was heterozygote (G/C), among them 2 parent ewes, 2 replacement rams and 12 ewes in the herd. The remaining 67.2% tested animals (41/61) were wild-type (G/G) (Fig. 5). The results are summarized in Table 1. Molecular testing by PCR of serum samples from the affected lambs showed negative results for Bluetongue virus and Pestivirus.

The diagnosis of autosomal recessive hereditary microphthalmia was established based on clinical, pathological and genetic evaluation of affected sheep. The clinical and pathological features, especially of the lamb subjected to the necropsy examination, were similar to those observed by Labs (1977), Roe et al. (2003) and Van der Linde-Sipman et al. (2003). All the affected sheep were homozygous for the c.338G > C SNP in the *PITX3* gene, confirming the occurrence of OMO in this outbreak.

A significant proportion of the herd animals collected for analysis (16/61) were identified as carriers of the microphthalmia SNP (14 ewes, two the dams of affected lambs). As only the two breeding rams recently introduced in the farm were carriers of this mutation, one or both could be parent of the affected lambs.

Although native females of the herd heterozygous for the c.338G > C SNP in the *PITX3* gene were already present, the rams used in the crosses were free from the microphthalmia SNP, explaining the absence of clinical disease until the introduction of the two new carriers rams. In this case, the introduction of animals who are carriers of the microphthalmia SNP therefore raises an important concern about dissemination of the mutant allele in the population of sheep in this herd (Tetens et al., 2007; Scott, 2012).

The occurrence of OMO and its degree of dissemination are not known in the population of Texel sheep in Brazil. However, it is known that congenital malformations are not infrequent conditions in sheep flocks (Dantas et al., 2010; Scott, 2012; Williams, 2010). These conditions are often not widely publicized by producers because of the associated financial impact (Scott, 2012). In addition, this could

[1] BD Vacutainer®.
[2] GE Healthcare Life Sciences, Buckinghamshire, England.
[3] Thermo Scientific™, DE, USA.
[4] Sigma-Aldrich®, MO, USA.
[5] Gene Codes, MI, USA.

Fig. 2. Eyeball of a normal lamb (A) and eyeball of a lamb with microphthalmia (B). The affected eye is 1.8 × 1.4 cm, there is no distinction between the anterior and posterior chambers, the vitreous cavity is filled with a white mass and presents a small, firm central structure of 0.4 cm surrounded by a blackened halo. The eyelids are practically occluding the eyeball.

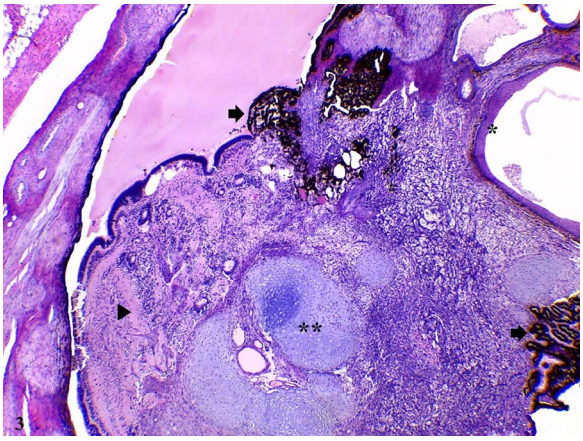

Fig. 3. Eye of a lamb with microphthalmia. Central area of the eye filled by mesenchymal tissue. The tissue proliferation is composed of spindle cells, sometimes with vesicular epithelial formations delimited by squamous epithelium (*), discrete and occasional proliferation of well-differentiated chondrocyte nodules (**) and undifferentiated nerve tissue (arrowhead). Retinal detachment with hypertrophy and disorganization of the pigment epithelium can also be noted (arrow). Hematoxylin and eosin stain. Obj. 100×.

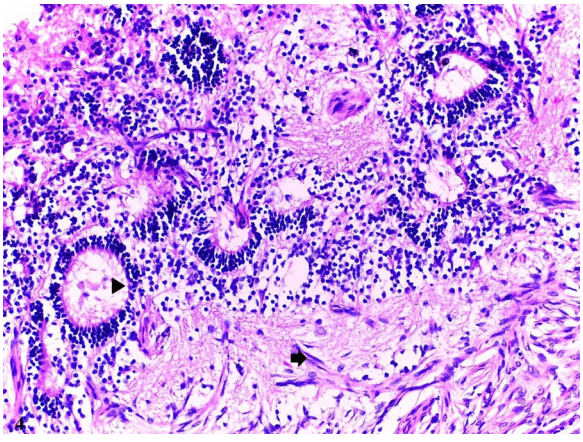

Fig. 4. Eye of a lamb with microphthalmia. Mesenchymal tissue of the central area of the eye composed of photoreceptor cells, sometimes forming rosettes (arrowhead). Part of the tissue is composed of spindle cells (arrow). Hematoxylin and eosin stain. Obj. 400×.

contribute to the lack of information on the number of OMO-affected lambs in herds (Drögemüller, 2010).

The recessively inherited condition of some diseases, as in OMO, contributes to increase the spread of disease silently through animal carriers of the mutant allele, since they do not exhibit any clinical alterations (Scott, 2012). Therefore, the detection and elimination of carriers is the main key for eradicating the mutant allele responsible for the disease. Caution should also be used in the purchase of breeding animals in locations where there is a known disease record (Roe et al., 2003; Tetens and Drögemüller, 2007).

Certain infectious and toxic conditions, especially in Brazil, should be considered as differential diagnoses of OMO. Bluetongue virus and Pestivirus infection which can promote defects during fetal development such as retinal dysplasia, microphthalmia and cataracts (Sawyer et al., 1991; Linklater and Smith, 1993). The affected necropsied sheep in this study was negative for infection with these viruses through PCR testing. In addition, there was no previous history of abortion or other clinical signs consistent with these disorders. In Brazil, microphthalmia also has been described as a result of ingestion of *Mimosa tenuiflora* during the gestation period, a toxic plant available in the semi-arid Northeast, not being found in the south of the country (Dantas et al., 2010). Other conditions such as congenital entropion of the eyelids exhibit a similar gross appearance; however, the eyeballs are of normal size, and there is no blindness (Drögemüller, 2010).

4. Conclusion

Hereditary microphthalmia associated with a mutation in the *PITX3* gene was diagnosed in this occasion based on clinical, pathological and genetic evaluation. A comprehensive study on the prevalence of the mutant allele in Texel sheep in the country still needs to be performed in order to determine the proportion of animals potentially able to spread OMO in the population of Brazilian Texel sheep.

The result of this study is a warning to veterinarians and farmers, especially in regions potentially involved in the production chain of the sheep slaughter industry, highlighting the importance of considering this disease in the differential diagnosis of congenital diseases in herds.

Conflict of interest

The authors declare no potential conflicts of interest.

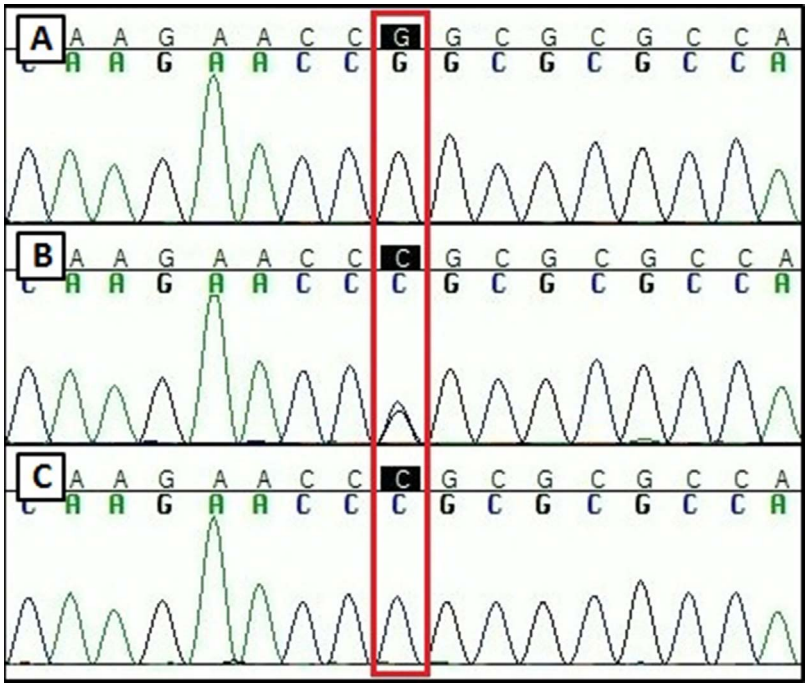

Fig. 5. A partial chromatogram obtained from the assembly of *PITX3* gene sequences from wild-type (A), carrier (B) and an affected (C) sheep. The normal G nucleotide shown in the picture was observed in the wild-type *PITX3* sequence. A double peak (G/C) was observed in the heterozygote sequence, and a point mutation (G > C) was observed in the OMO sequence (red box). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Determination of single nucleotide polymorphism c.338G > C in *Paired-Like Homeodomain 3* gene in a Texel herd.

Animal	Sequencing	Animal	Sequencing	Animal	Sequencing
Affected lamb 1	[a]C/C	Ewe 14	Wild Type	Ewe 34	Wild Type
Affected lamb 2	C/C	Ewe 15	Wild Type	Ewe 35	Wild Type
Affected lamb 3	C/C	Ewe 16	Wild Type	Ewe 36	Wild Type
Affected lamb 4	C/C	Ewe 17	Wild Type	Ewe 37	Wild Type
Ewe parent 1	[b]G/C	Ewe 18	G/C	Ewe 38	G/C
Ewe parent 2	G/C	Ewe 19	Wild Type	Ewe 39	Wild Type
Replacement ram 1	G/C	Ewe 20	Wild Type	Ewe 40	Wild Type
Replacement ram 2	G/C	Ewe 21	G/C	Ewe 41	Wild Type
[‡]Ewe 1	[†]Wild Type	Ewe 22	Wild Type	Ewe 42	Wild Type
Ewe 2	Wild Type	Ewe 23	Wild Type	Ewe 43	G/C
Ewe 3	Wild Type	Ewe 24	Wild Type	Ewe 44	G/C
Ewe 4	Wild Type	Ewe 25	Wild Type	Ewe 45	G/C
Ewe 5	Wild Type	Ewe 26	G/C	Ewe 46	Wild Type
Ewe 6	G/C	Ewe 27	Wild Type	Ewe 47	Wild Type
Ewe 7	Wild Type	Ewe 28	Wild Type	Ewe 48	G/C
Ewe 8	Wild Type	Ewe 29	Wild Type	[§]Ram 1	Wild Type
Ewe 9	G/C	Ewe 30	G/C	Ram 2	Wild Type
Ewe 10	Wild Type	Ewe 31	Wild Type	Ram 3	Wild Type
Ewe 11	Wild Type	Ewe 32	Wild Type	Ram 4	Wild Type
Ewe 12	G/C	Ewe 33	Wild Type	Ram 5	Wild Type
Ewe 13	Wild Type				

[a] Homozygosity.
[b] Heterozygosity.
[†] no carrier of the SNP to microphthalmia.
[‡] ewes 1–48: herd animals chosen at random for testing.
[§] rams 1–5: rest of the rams from farm.

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