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Infectious pathogens potentially transmitted by semen of the black variety of the Manchega sheep breed: Health constraints for conservation purposes

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ABSTRACT

Conservation of genetic resources from endangered breeds may be conducted through germinal banks. Preservation of healthy samples is paramount to avoid preserving pathogens shed with germinal products. The black variety of Manchega sheep (BMS), and endangered breed endemic to south-central Spain, is the subject of a conservation program; a germinal bank has been recently established. However, several pathogens circulating in BMS flocks may be shed with semen and threaten BMS preservation. Therefore, we investigated the sanitary status of BMS flocks and semen samples from 4 of the 17 flocks in which this variety is bred worldwide. A serological screening for Maedi-Visna virus, bluetongue virus, *Pestivirus* spp., *Brucella* spp., *Coxiella burnetii*, *Chlamydophila* spp., *Mycobacterium tuberculosis* complex, *Mycobacterium avium paratuberculosis*, *Anaplasma* spp., *Mycoplasma agalactiae*, *Toxoplasma gondii* and *Neospora caninum* was performed to assess for pathogens potentially shed by semen. Semen samples from 11 of the 35 BMS rams and 4 samples from coexisting rams of the white variety (WMS) were analyzed by PCR to detect Maedi-Visna virus, *C. burnetii*, *Anaplasma marginale*, *Anaplasma phagocytophilum* and *T. gondii*. Maedi-Visna virus RNA was detected in 3 semen samples (2 BMS and 1 WMS) while *C. burnetii* DNA was detected in 3 samples from WMS rams. Pathogens that can be transmitted by semen were present in BMS flocks, and Maedi-Visna virus and *C. burnetii* showed the highest potential for transmission by artificial insemination. Our results point to the need of testing semen samples kept for conservation purposes of BMS before using them for artificial insemination.

1. Introduction

Conservation of genetic resources from endangered species was set up in the 1970s (Taberlet et al., 2008) on the basis of the high economic relevance of regional breeds (Molina, 2010). The inverse relationship between rusticity and productivity ended in a significant reduction of autochthonous breeds and reduced their genetic diversity (Taberlet et al., 2011). However, global warming may drastically reduce water and food availability in the near future, consequently affecting the productivity of highly productive breeds. Autochthonous breeds would be better adapted to overcome extreme conditions, constituting an

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Table 1
ELISA tests employed for serological analyses of sheep serum samples.

Pathogen	Commercial ELISA	Producer/reference
Maedi-Visna virus	Elitest MVV/CAEV ELISA	Hyphen Biomed
Bluetongue virus	INgezim BTV Compac	Ingenasa
Pestivirus spp.	HerdChek BVDV Total Ab Test	IDEXX
Brucella spp.	INgezim Brucella Small Ruminants	Ingenasa
Coxiella burnetii	Ruminant Q Fever – Serum/Milk ELISA	Life Technologies
Chlamydophila spp.	Chlamydiosis Total Ab Test	IDEXX
Mycobacterium tuberculosis complex	In-house indirect ELISA	Boadella et al. (2012)
M. avium paratuberculosis	In-house indirect ELISA	Reyes-García et al. (2008)
Anaplasma spp.	Anaplasma Antibody Test Kit	VMRD
Mycoplasma agalactiae	*M. agalactiae* Screening Ab Test	IDEXX
Toxoplasma gondii	Toxotest Ab	IDEXX
Neospora caninum	*N. caninum* Antibody Test Kit cELISA	VMRD

important resource for humans (Hoffmann, 2010). Therefore, autochthonous breeds should be maintained as a resource for future generations.

The black variety of the Manchega sheep (BMS; *Ovis aries*) is an endangered breed native to the Autonomous Community of Castilla – La Mancha (CLM), in south-central Spain (MAGRAMA, 2012). The Manchega sheep is a rustic species that takes profit of natural resources to produce milk (cheese), meat (lambs) and wool (González et al., 1998) and represents a significant outcome source for rural economies in the region (Ramalho Ribeiro et al., 2006). However, according to official data from the Spanish Ministry for Agriculture, the BMS displays a decreasing population trend, with only 3820 individuals (3785 females and 35 males) in CLM (MAGRAMA, 2012). This situation prompted Spanish Authorities to preserve genetic resources from BMS when the demographic status of this variety was even worse (European Directive 2078/92).

A way to preserve the genetic diversity is establishing germinal banks in which sperm and oocytes may be preserved for the future (Roldán et al., 2006). However, sperm conservation may carry over preserving infectious pathogens shed with sperm that are maintained for a long time until being transmitted through artificial insemination (Philpott, 1993). Many pathogens shed by semen may induce severe reproductive failure in females and be counterproductive for autochthonous breed conservation programs (Santiago-Moreno et al., 2011). The most relevant pathogens that may be transmitted with sperm are listed in chapter 4.6 of the Terrestrial Animal Health Code of the World Animal Health Organization (http://www.oie.int/index.php?id=169&L=2&htmfile=chapitre_1.4.6.htm), including for ovine: Peste des petits ruminants virus, Maedi-Visna virus, bluetongue virus, scrapie, *Brucella melitensis*, *Brucella ovis*, *Mycoplasma agalactiae*, *Mycobacterium avium paratuberculosis* and *Mycobacterium tuberculosis* complex. However, other non-listed pathogens may be detected in sperm, such as *Chlamydophila abortus*, *Coxiella burnetii*, *Anaplasma* spp., *Toxoplasma gondii* and *Neospora caninum* (de Moraes et al., 2010; López-Olvera et al., 2009; Panadero et al., 2010; Santiago-Moreno et al., 2011) and they could be potentially transmitted to susceptible ewes. Currently there is no information on the sanitary risks associated to artificial insemination with BMS sperm from seminal banks. Therefore, we aimed to assess the presence and prevalence of pathogens in semen samples from the BMS seminal bank through molecular analyses.

2. Materials and methods

2.1. Flock selection

We selected four Manchega sheep flocks located in the province of Ciudad Real, southwestern Spain, where BMS is bred. One of the flocks corresponded to the reference flock for the Manchega sheep breed (CER), where both black and white (WMS) varieties are maintained and studied for productivity. The other three flocks were commercial flocks of the BMS that belong to the national association of breeders of the Manchega sheep (AGRAMA). Each flock owner/manager was interviewed to get information on the health protocols applied in the flocks.

2.2. Serological analyses

Between October and November 2011 blood was collected from the jugular vein of 45 animals per flock ($n = 180$; 17 males – 13 BMS and 4 WMS – and 163 females – all BMS). Blood was collected into sterile tubes without anticoagulant and transported under cool conditions to the laboratory, where it was centrifuged at $1500 \times g$ for 10′ to obtain the serum. Sera were preserved at −20 °C until serological analyses were performed. Serum samples were analyzed by ELISA to detect the presence of antibodies against 12 pathogens (Table 1), which were selected on the basis of their sanitary relevance and their inclusion in chapter 4 of the Terrestrial Animal Health Code of the World Animal Health Organization (http://www.oie.int/index.php?id=169&L=2&htmfile=chapitre_1.4.6.htm). For analyses, we followed the protocols established by manufacturers.

2.3. Collection of seminal samples

In April 2012, 15 rams – 11 BMS and 4 WMS – from 3 of the studied flocks were submitted to electro-ejaculation in the framework of a project to create a seminal bank of the Manchega sheep. Fifteen minutes before electro-ejaculation, males received an intramuscular injection of xylazine (0.2 mg kg^{-1} body weight; Rompun 2%; Bayer S.A., Barcelona, Spain). The rectum was cleaned of feces, and the