Journal of Animal and Veterinary Advances 7 (7): 812-815, 2008
ISSN: 1680-5593

Vesiculoseminitis and Ampullitis Caused by *Actinobacillus seminis* in a Pelibuey Breed Ram

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Abstract: A routine andrology and semen examination was carried out on a group of 12, one-year old, Pelibuey breed rams, but one individual had persisting leukocytospermia. No clinical alterations of the reproductive system were found in any of the rams. At slaughter of the problem animal, the seminal vesicles were found to be markedly lobulated and larger than normal, as were the vas deferens ampoules. When dissected, both structures contained creamy white yellowish material. Histopathology demonstrated ampullitis and seminal vesiculitis. A Gram-negative pleomorphic bacillus was isolated from both of the organs, which was characterized through the API 20E system and PCR. It was found to behave similar to the reference strain for *Actinobacillus seminis* ATCC 15768.

Key words: *Actinobacillus seminis*, ampullitis, vesiculitis, ram

INTRODUCTION

The cases of epididymitis in rams are mainly associated with infections caused by *Brucella ovis* or *Actinobacillus seminis*, which can be related or not to pathologies of associated glands, nevertheless other microorganisms could be responsible for these pathologies (Burgess, 1982), especially Gram-negative pleomorphic bacilli such as *Actinobacillus actinomycetencomitans*, *Histophilus somni* and *Mannhemia haemolytica*, whose identification can be difficult. Systems such as API 20E (Erasmus, 1983), API ZYM (Cousins and Lloyd, 1983), as well as electrophoresis of outer membrane proteins (Stephens *et al*., 1983), digestion with restriction enzymes (Mc Gillivery and Webber, 1989) and more recently PCR (Appuhamy *et al*., 1998) can be used for their characterization. Even though the main pathology in the reproductive system of rams is epididymitis, it is frequently associated with alterations of the associated glands of the reproductive system (Searson, 1986; Foster *et al*., 1987). The few descriptions in the literature of the alterations of the associated glands could be due to their location within the pelvis which in itself impedes their inspection in live animals and making slaughter necessary for their examination or the application of more costly techniques such as ultrasound (Ahmad *et al*., 1993).

In experimental infections with *A. seminis* by different pathways, the persistence of the bacterium has been noted in the associated glands, even in cases where epididymitis could not be reproduced (Acosta *et al*., 2006; Al-Katib and Dennis, 2005). Furthermore, the presence of transitory leukocytospermia in ram's semen has been described together with the isolation of bacteria from normal associated glands (Jansen, 1980, 1983)

In the present study we describe one case of persistent leukocytospermia, without epididymal alterations, in a one-year old Pelibuey breed ram that had lesions in vas deferens ampoules and seminal vesicles.

MATERIALS AND METHODS

An andrology clinical examination was carried out on a group of 12 one-year old Pelibuey breed rams while placed in a "sitting" position. We measured the testicular diameter and semen was obtained by electroejaculation

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with the animals placed in lateral decubitus position and extended penis. The sampling was carried out for 8 weeks. Bacteriology tests were carried out on the semen. It was determined the volume of the ejaculate and sperm concentration (with a hemocytometer), as well as sperm alterations with a Giemsa-stained slide (Hafez, 1993). It was counted the number of Polymorphonuclear cells (PMN) in three microscope fields under 40X power (Kott *et al*., 1988). Immediately after they were obtained, the semen samples were inoculated by swab in blood agar and then incubated at 37°C during 24-48 h with a 10% CO_2 environment. The isolates were compared to the ATCC 15768 strain of *A. seminis* through the API E20 method (Erasmus, 1983).

The animal that had leukocytospermia was slaughtered and samples were obtained for histopathology and bacteriology from the associated glands, epididymis (head and tail) as well as from both testicles.

In order to obtain a definitive identification of the strains isolated from semen and the reproductive system we extracted DNA from them (Ausubel *et al*., 1995) to carry out PCR (polymerase chain reaction) identification by using the primers designed by Appuhamy *et al*. (1998) for the 16S-23S ribosomal spacer that have the following sequences 3´AAGAAAAAGACGAAGAGACATT5´ and 3´CTTATCTTTCTTAAGCCCTGAC5´. The reaction was carried out in a 25 μL final volume that contained 2.5 μL of PCR buffering solution (100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3) 2 μL (0.2 μM) of each dNTP (Boheringer Mannheim), 1.0 μL (50 pM) of each primer, 0.125 μL (0.625 U) of Taq polymerase (Biotecnologias Universitarias UNAM), 2.5 μL DNA and 16.87 μL of sterile milli-Q water. Amplification was carried out in a GeneAmp PCR system 2400 (Perkin Elmer) under the following program: initial denaturing step at 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec, with a final extension step at 72°C for 6 min. The PCR products were run in a 1% agarose gel stained with ethidium bromide.

RESULTS

The problem animal was within those that had the greatest testicular diameter, an average of 36.1±0.7 cm from 8 measurements taken and averaged the greatest ejaculate volume. Nevertheless, the semen had the greatest percentage of abnormalities with abundant PMN.

At clinical inspection the problem animal did not show any alterations in the testicles or epididymis; both sides were symmetrical, moved normally within the scrotum and had a normal elasticity and consistency. Nevertheless, semen samples had visible glutinous material (floccules) and bloody appearance. The ejaculate volume was abundant and variable, 1.6±0.9 mL, with a sperm concentration of 2.1×10^9 sperm mL^{-1}. The Giemsa-stained slides from semen demonstrated high quantities of PMN that appeared agglomerated within many of the observation fields making their count impossible, nevertheless in those that the count was possible we found between 22-40 PMN per field (40X). The average percentage of sperm abnormalities was 21±4.8, with the majority of them classed as secondary: bent tails and loose heads.

When the ram was slaughtered the seminal vesicles were found to have highly apparent lobulation and size increase, the same as the vas deferens ampoules. Both glands when dissected had creamy white-yellowish material. No alterations were found in the epididymis and the testicles. Pure isolates of the same bacteria found in the semen could be obtained directly from the ampoules and seminal vesicle.

Epididymis, testicles and bulbourethral glands did not show alteration at histopathology examination. Nevertheless, ampullitis with lymphoid interstitial infiltration, sometimes with nodular appearance, could be seen in the mucosa of the vas deferens, as well as presence of PMN in the interior of the acini (Fig. 1). The seminal vesicles presented interstitial lymphoid infiltration with the presence of protein exudates and PMN in the interior of the acini.

Semen bacteriology gave pure cultures of large white-grey colonies that when Gram-stained slides of them were seen under the microscope revealed the presence of pleomorphic Gram-negative bacilli. With the API E20 method they were compared against the ATCC

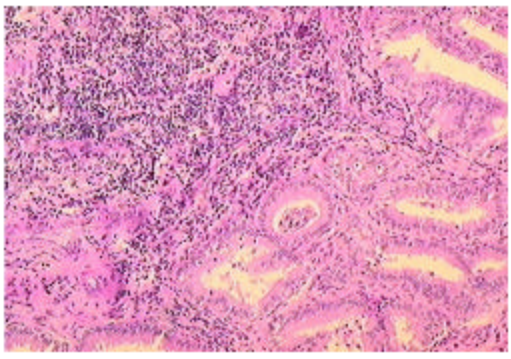

Fig. 1: Appearance of the Ampullae with interstitial lymphoid infiltration with nodular appearance. 10X

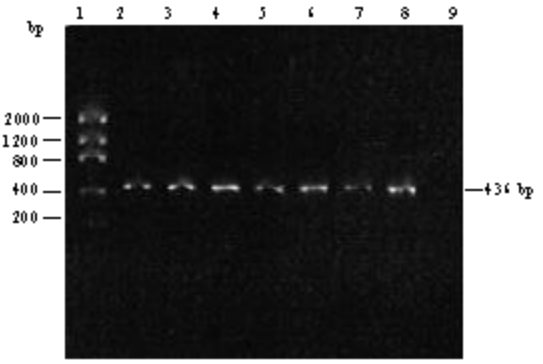

Fig. 2: Electrophoresis of 16S-26S ribosomal spacer Lane 1: MW Molecular weight ladder, 2 *Actinobacillus seminis* ATCC 15768, 3-6 semen isolates (8, 11, 12, 13), 7 ampullae isolate, 8 seminal vesicle isolate, 9 negative

type strain of *A seminis* (Erasmus, 1983) with similar results. The isolates were characterized as catalase, oxidase, ornithine and nitrate positive and had weak reactions in glucose, mannose and inositol, while some of the isolates reacted to arabinose. The PCR results showed evidence that all samples, four semen isolates (8, 11, 12 and 13) as well as the isolates from the seminal vesicles and vas deferens ampoules, amplified a 436 bp band similar to the ATCC 15768 reference strain (Fig. 2).

DISCUSSION

The results from bacteriology were comparable to those from the strains of *A seminis* that Erasmus (1983) presented, that had weak reactions to glucose, mannose, inositol and arabinose and are clearly different from the *Manhemia haemolytica* strains analyzed by the same author.

Although, the pathologies of the reproductive system of sheep are generally associated with cases of epididymitis due to *B. ovis* and *A. seminis*, there are pathologies of the associated glands that are not necessarily related to these pathogens or to the epididymitis condition. Leukocytospermia has also been described as transitory in semen from rams, as well as the presence of bacteria from associated glands without any pathology conditions present (Jansen 1980, 1983). In the case of *B. ovis* a correlation has been described between the presence of white cells and the presence of loose heads in the semen of animals with subclinical infection (Kimberling *et al*., 1986). In this sense a high number of secondary alterations was found, 21±4.8, in terms of loose head and bent tails, besides of the presence of inflammatory cells in the semen. For this reason this animal is questionable for the reproduction (Bagley *et al*., 1984). It is worth noting that carrying out sperm count is difficult when PMN are highly abundant and the sperm are agglutinated.

CONCLUSION

It was conclude that the origin of the PMN were the lesions observed in the ampoules and seminal vesicles, without the presence of any other type of alteration in the reproductive system. The presence of this type of alterations cannot be seen in a routine andrology clinical examination and sometimes, as it happens in this case, the animal could have been selected for reproduction by this procedure. Nevertheless, the collection of semen demonstrated visible abnormalities in the ejaculate and showed the presence of a high quantity of sperm abnormalities and PMN, which are considered to indicate low fertility of the ram.

ACKNOWLEDGEMENT

To MVZ German Garrido for carrying out the histological preparations. This project was funded in part with funds from PAPIIT-UNAM: IN206101

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