

Research Paper

Isolation of *Actinobacillus seminis* from a goat with clinical epididymo-orchitis in Brazil

Fabrine Alexandre dos Santos¹, Edísio Oliveira de Azevedo¹,
Sérgio Santos de Azevedo¹, Felício Garino Júnior¹, Rinaldo Aparecido Mota²,
Pomy de Cássia Peixoto Kim², Ana Lisa Vale Gomes², Clebert José Alves¹

¹Unidade Acadêmica de Medicina Veterinária, Centro de Saúde e Tecnologia Rural,
Universidade Federal de Campina Grande, Patos, PB, Brazil.

²Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil.

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Abstract

The present study reports the first isolation of *Actinobacillus seminis* from a goat in Brazil. A four-year-old Moxotó breeding goat in a flock of 70 goats and 65 sheep reared together in the county of Patos, semiarid region of Northeastern Brazil, showed clinical signs of unilateral orchitis and epididymitis. Diagnosis of *A. seminis* infection was confirmed by association of clinical findings, bacterial isolation and 16S rRNA gene sequencing. This result suggests that *A. seminis* may be an additional cause of infertility in goats, and that sheep may be the source of infection because the mixed farming system allows the contact between sheep and goats in the semiarid region of Northeastern Brazil.

Key words: epididymitis, goats, *Actinobacillus seminis*, isolation.

Introduction

Goat breeding has great economic importance in many countries, including Brazil. However, several factors negatively affect goat breeding including nutritional and health issues, and infectious diseases (Pinheiro *et al.*, 2000).

It is documented that ovine epididymitis due to *A. seminis* is one of the major cause of economic losses as it reduces and interferes with the fertility of infected ram. Its pathogenesis is uncertain, but it is suggested that *A. seminis* may ascend from the preputial cavity and colonizes the genital tract, causing the clinical signs, and that predisposing factors such as stress induced by hormone changes, as well as nutritional deficiencies can cause the development of orchitis and epididymitis, particularly in young sheep (Hajtós *et al.*, 1987; Dibarrat *et al.*, 2006).

The first isolation of *A. seminis* was reported by Baynes and Simmons (1960), in Australia, in semen of rams with epididymitis. Then, the bacterium was isolated in several occasions in different countries: United States of America (Livingston and Hardy, 1964), South Africa

(Worthington and Bosman, 1968), New Zealand (Bruere *et al.*, 1977), Hungary (Hajtós *et al.*, 1987), Argentine (Robles *et al.*, 1990), Brazil (Gomes *et al.*, 2001; Gregory *et al.*, 2009; Bezerra *et al.*, 2012), United Kingdom (Heath *et al.*, 1991), Spain (Puente-Redondo *et al.*, 2000), Turkey (Diker *et al.*, 1991), Kenya (Mbai *et al.*, 1996) and Mexico (Narez *et al.*, 1999). Interestingly, it was reported that *A. seminis* had been isolated from bulls and goat in South Africa (Van Tonder and Bolton, 1970; Van Tonder, 1973) and from cattle in New Zealand (Dixon *et al.*, 1983).

This paper documents the first isolation of the microorganism from a goat with clinical signs of epididymitis and orchitis in Brazil.

Materials and Methods

Field observation and clinical signs

During a visit in a farm in the county of Patos, State of Paraíba, semiarid region of Brazil to investigate the reasons of reduced fertility, where there were 135 animals, 70 goats and 65 sheep reared, a four-year-old Moxotó breeding goat

was found to have clinical signs of unilateral orchitis and epididymitis on the right side (Figure 1). Clinical examination of the area showed firm consistency of the epididymis and testis, with absence of ulceration or nodularity, sensitivity to palpation and adhesions of the scrotum.

Serological examination

Blood samples were collected from all animals by jugular vein puncture. Agar gel immunodiffusion (AGID) test was used for the detection of anti-*B. ovis* antibodies with commercial kits produced by the Institute of Technology of Paraná (TECPAR, Curitiba, Paraná, Brazil), using lipopolysaccharide antigen and proteins of *B. ovis* strain Reo 198. Buffered Acidified Plate Antigen Test (BAPA) was used for serodiagnosis of *B. abortus* using an inactivated *B. abortus* strain 1119-3 antigen, produced by the Institute of Technology of Paraná (TECPAR, Curitiba, Paraná, Brazil), at the cellular concentration of 4% and Rose Bengal stained (OIE, 2008).

Bacteriological examination

Weekly samples of semen were collected from the goat diagnosed with epididymitis and orchitis using electroejaculation for three successive weeks. Fine needle aspirates of a caseous and white to yellow material of the testis and the tail of the epididymis were also performed by aspiration with disposable syringes and needles. The samples were plated on blood agar and *Brucella* agar (Difco, Franklin Lakes, NJ, USA) enriched with defibrinated sheep blood at a concentration of 5% of the total volume. All samples were incubated at 37 °C in an atmosphere containing 10% of carbon dioxide (CO₂). Cultures were examined daily for five days for visible growth. The bacteria isolated were subjected to Gram staining and biochemical tests (catalase, oxidase, nitrate and esculin; and acid production from maltose, xylose, galactose and trehalose) according to Krieg and Holt (1984).

Molecular examination

Semen, aspirates and isolated bacteria samples were subjected to DNA extraction according to the manufacturer's protocol using the commercial kit "Qiagen DNeasy® Blood and Tissue Kit" (Qiagen, Austin, TX, USA).



Figure 1 - Four-year-old Moxotó breeding goat with clinical signs of unilateral orchitis and epididymitis.

Polimerase Chain Reaction (PCR) technique for *Brucella* spp.

Specific primers directed to the 16S-23S ribosomal RNA (rRNA) interspace region of *Brucella* spp. (ITS66: ACATAGATCGCAGGCCAGTCA and ITS279: AGATACCGACGCAAACGCTAC) (Keid *et al.*, 2007) were used, which amplify with 214 bp. The amplification reaction mixture was prepared in a volume of 12.5 µL containing 200 µM of each deoxynucleoside triphosphate, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl₂, 0.5 mM of each primer, 1.5 U platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 5 µL of template DNA.

The reaction was performed in a DNA thermal cycler (MJ Research PTC 200 DNA engine, Watertown, MA, USA) without mineral oil. Ultrapure water was used as negative control while *B. ovis* strain 63/290 served as positive control. After an initial denaturation at 95 °C for 2 min, the PCR profile was set as follows: 30 s of template denaturation at 95 °C, 30 s of primer annealing at 62 °C and 30 s of primer extension at 72 °C, for a total of 40 cycles, with a final extension at 72 °C for 5 min.

PCR technique for *A. seminis*

SRJAS1 (CTTATCTTTCTTAAGCCCTGAC) and SRJAS2 (AAGAAAAAGACGAAGAGACATT) primers were used, which amplify the 16S rRNA gene with 436 bp (Appuhamy *et al.*, 1998b). Amplification reactions were performed in a final volume of 25 µL containing 2.5 µL of PCR buffering solution (100 mM Tris-HCL, 15 mM MgCl₂, 500 mM KCl, pH 8.3), 2 µL (0.2 µM) of each dNTP, 1.0 µL (50 pM) of each primer, 0.125 µL (0.625 U) of Taq polymerase (Qiagen, Austin, TX, USA), 2.5 µL of genomic DNA and 16.87 µL of Milli-Q ultrapure water, according to the supplier's protocol.

The thermal profile of the stages of reactions was measured on Thermal Cycler (Bioer Technology CO LTD, Hangzhou, China), consisting of an initial denaturation of DNA at 94 °C (1 min) and followed by 35 cycles at 94 °C for 30 s for denaturation, 55 °C for 30 s for annealing, 72 °C for 6 min for extension and final extension of 1 min at 72 °C, according to Appuhamy *et al.* (1998a).

Amplified products were analyzed by electrophoresis in a 2% agarose gel and then stained with ethidium bromide (0.5 mg/mL). The DNA bands were visualized under UV light.

DNA sequencing

Sequencing reactions were performed using the kit "The BigDye® Terminator v3.1 Cycle Sequencing" (Applied Biosystems, Foster City, CA, USA) and polymerization conditions were performed in 96 well plates according to manufacturer's instructions. Samples were sequenced by the dideoxy terminator method in automatic in ABI PRISM

3100 Genetic Analyser for automated sequencing (Applied Biosystem), with subsequent analysis of sequence identity performed using Jalview software (<http://www.jalview.org/>).

Results

Serological findings

All animals were found to be negative (100%) for *B. abortus* by BAPA test, while five sheep (n = 5, 8%) and nine goats (n = 9, 13%) including the diseased goat were found to be positive for *B. ovis* by IDGA.

Bacteriological findings

Small smooth, shining and non-pigmented colonies (1 to 2 mm) were isolated from semen samples. They appeared to be non-motile, Gram negative coccobacilli. Biochemically, isolated organism was catalase, oxidase, nitrate and esculin positive. It produces acid from maltose and xylose but not from galactose and trehalose.

PCR findings

PCR was negative for *Brucella* spp. However, *A. seminis* DNA was amplified from weekly-collected semen samples and isolated colonies (isolate SAAS01). Sequence of PCR products from semen samples and colonies that revealed a DNA fragment of a standard size showed 99% similarity with the region of the 16S rRNA gene of *A. seminis*.

Discussion

Positive sequencing following isolation of *A. seminis* in goats is the first report of this kind in Brazil. A fact that deserves attention concerns the mixed farming system of goats and sheep in Brazil. The isolation of *A. seminis* from a Boer goat, bull semen, and from an Angora goat, an Afrikaner and Friesland bull raised the wide range of host involvement and the role of both sheep and goat in the dissemination of actinobacillosis (Al-Katib and Dennis, 2005) and indicate that these species may serve as sources of infection for *A. seminis* and thus aid in the spread and transmission of infection when they are in contact with rams under intensive systems (Al-Katib and Dennis, 2009).

Clinical and pathological findings of the epididymitis and orchitis in the tested goat were properly similar to those described by others (Bezerra *et al.*, 2012). The signs of infection by *A. seminis* deserve special consideration because the effects are not noticeable or measurable (Gomes *et al.*, 2001), particularly in extensive breeding systems or when farmers are not aware of the economic importance of the disease (Bezerra *et al.*, 2012). It should be stressed that the clinical changes observed are usually associated with low concentration, low motility and non viability of sperms, besides the presence of neutrophils in the semen, which affect the fertility rate of breeders, and in the regions where the

disease has not been previously diagnosed, economic losses may be even greater (Baynes and Simmons, 1960; Van Tonder, 1973; Bezerra *et al.*, 2012).

Our findings indicated that serological tests did not yield satisfactory results. In this study, positive AGID in the goat raises the possible simultaneous presence of *B. ovis* seropositive animal when infected with *A. seminis*, which should be subject of further investigations. This in contrast with those results reported by Gomes *et al.* (2001) and Gregory *et al.* (2009) that isolated *A. seminis* in sheep, which were AGID-negative for *B. ovis*.

In the present study, the isolation of *A. seminis* from a goat would suggest that infection be disseminated in adult breeding goats, which makes control difficult, because of the continued exchange of breeding goats between the farmers and their commercialization without proper healthcare.

The differential diagnosis of orchitis and epididymitis must be based on clinical and pathological diagnosis, the isolation and identification of the causative agent (Sponenberg *et al.*, 1983). Our findings indicated that the morphological, and biochemical characteristics allowed its identification as *A. seminis*.

It is concluded that *A. seminis* should be considered as differential diagnosis in cases of epididymitis in goats particularly in the Northeastern Brazil where the mixed farming system is widely adopted, and the agent has already been isolated in sheep.

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