

Isolation of *Histophilus somni* from the vaginal exudate of goats that have aborted

Aislamiento de *Histophilus somni* a partir de exudado vaginal de cabras que presentaron aborto

CÉSAR NOÉ BADILLA MEDINA¹, FRANCISCO AGUILAR ROMERO², ÉRIKA GABRIELA PALOMARES RESÉNDIZ², LUIS MACONETZIN ISIDRO REQUEJO³ Y EFRÉN DÍAZ APARICIO^{2*}

¹ Facultad de Medicina Veterinaria y Zootecnia - Universidad Autónoma de Sinaloa. Blvd. San Ángel s/n, Fracc. San Benito cp. 80236, Culiacán, Sinaloa, México.

² CENID Microbiología, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Carretera Federal México-Toluca Km. 15.5, Cuajimalpa, D. F., México. C.P. 05110.

³ Campo Experimental La Laguna Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Matamoros, Coahuila, México.

*Correo electrónico: efredia@yahoo.com

ABSTRACT

In order to determine the causative etiologic agent of abortion in goats that have had negative serological and bacteriologic responses to the most common causes of frequent abortions in Mexico, analyses were performed on 100 vaginal swab samples of adult goats with a history of abortion from Comarca Lagunera, a metropolitan area in Northwestern Mexico. The swabs were transported with Ames' medium with activated charcoal, inoculated in chocolate agar, supplemented with 10 percent defibrinated bovine blood and 0.5 percent yeast extract, and incubated from 24 to 48 hours at 37°C with CO₂ at a concentration of 10 percent. Tintorial affinity, biochemical tests, microscopic morphology and identification through a species-specific PCR were determined for each isolate. Two isolates were obtained and identified as *H. somni*, representing 2 percent of the total samples. This is the first report about the isolation of *H. somni* from the vaginal exudate of goats that have aborted.

Key words: Goats, *Histophilus somni*, abortion, Mexico

INTRODUCTION

Histophilus somni is a small, pleomorphic, Gram-negative coccobacillus bacterium that is oxidase positive and generally indol positive (García-Delgado et al. 1977; Angen et al. 2003).

Histophilus somni was first isolated in Colorado, USA in 1956, as a causative agent of encephalitis in bovines and later in cases of meningoencephalitis and thromboembolic meningoencephalitis (Kennedy et al. 1960; Bailie et al. 1966; Panciera et al. 1968). There has only been one report of its isolation in a vagina, but this was not a case with a history of abortion (Janosi et al. 2009). There is also another report describing the isolation of *H. somni* from nasal exudates in goats from the Mixteca region in Puebla, Mexico (Pérez-Romero et al. 2011). However, there has been no information about the presence of this microorganism as causative agent of abortion in goats.

RESUMEN

Con el objetivo de determinar el agente etiológico en cabras que habían presentado aborto, y que tenían resultados negativos en las pruebas serológicas y bacteriológicas a las enfermedades causales de aborto más comunes en México, se analizaron exudados vaginales de 100 cabras con historial de aborto procedentes de la Comarca Lagunera, región ubicada en el norte de México. Los exudados vaginales fueron colectados con hisopos que se insertaron en el medio de transporte Ames, las muestras fueron inoculadas en agar chocolate suplementado con 10% de sangre de bovino desfibrinada, y 0.5% de extracto de levadura, e incubados durante 24 a 48 horas a 37°C en una estufa bacteriológica con una concentración de CO₂ del 10%. Los resultados de la identificación que fueron realizados a los aislamientos, mediante la tinción de Gram, la morfología colonial observada al microscopio, de las pruebas bioquímicas, y de la identificación de la especie mediante PCR. Dio por resultado que dos aislamientos fueron identificados como *H. somni*, lo que representa el 2% del total de las muestras. Este es la primera notificación referente al aislamiento de *H. somni* a partir de cabras que habían presentado aborto.

Palabras clave: Cabras, *Histophilus somni*, aborto, México

Comarca Lagunera is an area located in the bordering region between the states of Durango and Coahuila, in Northern Mexico, and constitutes 44.7% of the national production of goat milk. It is an endemic area for caprine and bovine brucellosis, with rates of 62% among goat herds (Pérez-Romero et al. 2011). A study of leptospirosis seroprevalence found that 73.6% of the goats were positive (García, 2011). Seroprevalence for *Chlamydia abortus* was 19/54 (35%) in milking herds and 32/439 (7.28%) in sampled goats (Fernández, 2011). The purpose of this study was to establish the causative etiologic agent of abortions in goats from Comarca Lagunera Mexico, which had had negative serological and bacteriological responses to the most common causes of abortion in Mexico.

MATERIAL AND METHODS

Vaginal exudate was collected from 100 goats that had had an abortion in the previous 30 days or less. Their serological response to the diagnosis of brucellosis, chlamydiosis and leptospirosis was negative. In order to collect samples from this number of animals, sixty herds were examined in the following municipalities to Comarca Lagunera: 9 herds in Matamoros, 3 in Torreon, 15 in Viesca, 11 in Gomez Palacio, 2 in Tlahualilo, 11 in Francisco I Madero, and 12 in San Pedro.

The zootechnical purpose of goats is the production of milk, which is sold to cheese factories. These herds are raised extensively in common areas where the goats graze all day long. In the evening they are herded into a shared corral. They receive no supplements and are vaccinated with the Rev 1 *Brucella melitensis* vaccine.

Vaginal exudate samples were inoculated in blood agar, with 10% sheep blood and chocolate agar supplemented with 10% defibrinated bovine blood, and 0.5% yeast extract. They were then incubated at 37 °C, from 24 to 48 hours, with CO² at a concentration of 10% (Inzana and Corbeil, 1987; Humphrey, and Stephens, 1983).

After 48 hours of incubation, colonies were selected according to their morphological characteristics (Garcia-Delgado et al. 1977; Humphrey, and Stephens, 1983). Tintorial affinity, microscopic morphology, and identification with API 20 Microsystem (Bio Merieux) and a species-specific PCR were carried out for each isolate (Angen et al. 1998).

DNA extraction was performed using guanidine thiocyanate, according to the method described by Pitcher et al. (1989). Specific primers were used to amplify a fragment of 407 base pairs of ribosomal subunits 16S rRNA 5'-GAAGGCGATTAGTTTAAGAG-3', and 5'-TTCGGGCACCAAGTATTCA-3'. PCR Reaction was performed in a final 50-ml volume that contained 3 µl of MgCl₂ (25 mM); 5 µl of 10x buffer; (200 mM Tris pH 8.4, 500 mM KCl); 2 µl of 100 µM dNTP of each one; 100 pm of each of the primers; 0.5 U of Taq polymerase (Invitrogen®), and 5 µl (150 ng) of DNA.

Amplification conditions were as follows: denaturalization at 94 °C during 3 min, followed by 35 cycles a 94 °C during 1 min; alignment at 55 °C for 1 min and extension at 72 °C for 1 min. Amplification products were seen in an 1.2% agarose gel, stained with ethidium bromide (Angen et al. 1998). *H. somni* strain ATCC 2336 was included as positive control in the PCR reaction.

RESULTS

Only two isolates were obtained from the 100 swabs of vaginal isolate, with the following characteristics: convex and shiny colonies in circular shape, approximately 1-2 mm in diameter, with slightly yellowish pigment and buttery consistency. Pleomorphic Gram negative coccobacilli were observed at the microscope and were later confirmed by the API20 microsystem, thus leading to their identification as *H. somni*.

DNA was extracted from the isolate and from the *H. somni* ATCC 2336 strain used as control. A PCR product of 407 base pairs was amplified.

DISCUSSION

Although *H. somni* has been previously isolated twice in goats (Janosi et al. 2009; Pérez-Romero et al. 2011), there have been no reports of its isolation in goat abortions. However, there have been reports of abortion in cows due to *H. somni*. In a study on experimental infection in pregnant cows, in which five cows were intravenously inoculated with 5 x 10⁸ ufc of *Haemophilus somnus*, (former name of *H. somni*) one of the cows had an abortion within 5 days and another one reached full-term and gave birth to an apparently normal calf, but both cows excreted *H. somnus* in their vaginal exudates for seven weeks (Stuart et al. 1990).

Headley et al. (2013) determined in Brazil the presence of amplified DNA of *H. somni* by means of PCR in the brain of a fetus aborted with lesions suggestive of histophilosis.

Janosi et al. (2009) reported that the presence of *H. somni* in the membranes of the genital mucosa in goats was correlated to the estrus cycle and frequent contact with male

ovines, due to the fact that the sampled goats coexisted closely with ovines and that *H. somni* was only isolated from herds in these conditions. They also established that interspecies sexual activity of sheep during estrus season may explain the infection in goats. This was confirmed with the partial sequencing of the 16S rRNA gene from the isolated strain, verifying the sequences of *H. somni* obtained from animal species, as well as the identification, homology and high level of conformity of the metabolic fingerprints, which showed a common source of infection.

Comarca Lagunera is a semi desert region where the scarce vegetation, high temperatures, grazing conditions and long journeys to the grassland favor a majority of goats. The herd we studied was made up exclusively of goats, which eliminated the interspecies contagion factor that was present in the study by Janosi et al. (2009). In these grazing conditions, it is very complicated to collect fetuses or placentas, since abortions take place in most cases during the way back or stay in the grasslands, and the shepherd does not realize so until they return to the enclosure and he detects lochia or placental residues that indicate the goat was pregnant.

The presence of *H. somni* in this case does not seem to be a frequent problem, as it was isolated in only two cases from 100 vaginal exudates, leading to the conclusion that this is the first isolation worldwide of *H. somni* from samples of vaginal exudates in goats that had had an abortion.

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