DOI: 10.1590/1808-1657v71p3832004

SCIENTIFIC COMMUNICATION

ISOLATION OF *LEPTOSPIRA* SPP. FROM KIDNEYS OF SHEEP AT SLAUGHTER

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ABSTRACT

Attempts were made to isolate leptospires from kidneys of sheep slaughtered in the public slaughterhouse of the city of Patos, Paraíba state, Northeast region of Brazil. Kidneys from 80 sheep without clinical signs of leptospirosis were used. The Ellinghausen-McCullough-Johnson-Harris (EMJH) medium modified with the addition of 10% rabbit serum was used for leptospira isolation. Renal tissue samples were taken by renal perforation with a sterile Pasteur pipette after superficial cauterization. The cultures were observed weekly by dark field microscopy for up to 16 weeks. With this methodology, *Leptospira* spp. could be isolated from four renal tissue samples.

KEY WORDS: Isolation, *Leptospira* spp., sheep, culture.

RESUMO

ISOLAMENTO DE *LEPTOSPIRA* SPP. DE RINS DE OVINOS ABATIDOS. Foram feitas tentativas de isolamento de leptospiras a partir de rins de ovinos abatidos no matadouro público do Município de Patos, Estado da Paraíba, região Nordeste do Brasil. Foram usados rins de 80 ovinos sem sinais clínicos de leptospirose. O meio de Ellinghausen-McCullough-Johnson-Harris (EMJH) modificado com a adição de 10% de soro de coelho foi usado para o isolamento. As amostras de tecido renal foram obtidas pela perfuração do órgão com uma pipeta Pasteur estéril após cauterização da superfície. Os cultivos foram observados semanalmente por microscopia de campo escuro durante 16 semanas. Com essa metodologia, foi isolada *Leptospira* spp. de quarto amostras de tecido renal.

PALAVRAS-CHAVE: Isolamento, *Leptospira* spp., ovinos, cultivo.

Leptospirosis is a worldwide zoonosis that causes elevated harm to animal production. Among animals of economic interest, sheep have high value in the Northeast region of Brazil. The main impact of the disease is the decrease of the reproductive performance of the affected herds (VASCONCELLOS, 1993). The outbreaks occur by exposure to water contaminated with urine or tissue from infected animals (FAINE et al., 1999). The slaughterhouse workers are mostly exposed to leptospires, as these organisms are frequently located in the kidney and bladder (FAINE et al., 1999).

The clinical diagnosis of leptospirosis is inconclusive due to different clinical signs that can be attributed to other pathogenic agents (VASCONCELLOS, 1993). The microscopic agglutination test (MAT) is considered the reference test among the several serological methods for leptospirosis diagnosis. However, isolation and identification of the microorganism allows for definitive diagnosis, and provides for epidemiological and prophylactic studies of this disease (FAINE et al., 1999).

The present study was designed to carry out isolation of leptospires from kidneys of slaughtered sheep.

The study was carried out between September 2000 and July 2001 in the Transmissible Diseases Laboratory at the Department of Veterinary Medicine of Campina Grande Federal University (UFCG) – Brazil. For the isolation attempts, kidneys from 80 sheep without clinical signs of leptospirosis slaughtered in the public slaughterhouse of the city of Patos, Paraíba state, Northeast region of Brazil, were used.

The kidneys (one from each sheep) were collected aseptically on the slaughterhouse eviscerating table, being separated in sterile polypropylene bags and

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transported to the laboratory under refrigerated conditions (4º C). In the laboratory, the kidneys were processed within 3-4 hours from the time of collection (THIERMANN, 1983). The kidneys were submitted to disinfection by submersion in 5% sodium hypochlorite for 20 minutes. The Ellinghausen-McCullough-Johnson-Harris medium (EMJH) (Difco-USA) was used for leptospira isolation modified with the addition of 10% rabbit serum enriched with calcium chloride and magnesium chloride (ALVES, 1995). This culture medium was prepared in two formulations, one without antibiotics and the other with the addition of 5-fluorouracil (400 mg / L; Sigma-USA).

For each kidney (n = 80), the renal capsule was removed and tissue samples were taken by perforation with a sterile Pasteur pipette after superficial cauterization (PASSOS et al., 1988) and cultured in duplicate in modified EMJH medium added with antibiotic (resulting in 160 cultured samples) and incubated at 28º C for 24h, followed by subculture in duplicate in the same culture medium but without antibiotic. Inoculates used for isolation and subcultures corresponded to 10% of the volume of the culture medium cultured. The cultures were observed weekly by dark field microscopy for up to 16 weeks (ELLIS et al., 1983). When the presence of leptospires was observed, a subculture in duplicate was carried out in modified EMJH medium without antibiotic. The tubes that presented contamination in the weekly assessment had a new subculture in modified EMJH with antibiotic and after 24h incubation at 28º C they were returned to the culture medium without antibiotic. For maintenance of leptospires isolated from ovine renal tissue, the semisolid Fletcher medium (Difco-USA) was also used.

Leptospires were isolated from four of the 160 sheep kidney samples cultured. Isolation surveys conducted in distinct countries showed the importance of sheep in the epidemiology of the leptospirosis. GORDON (1980), in Canada, isolated the serovar hardjo from a sheep with focal chronic interstitial nephritis in both kidneys. ELLIS et al. (1983), in Australia, isolated the serovars pomona, australis and hebdomadis by culture of sheep renal tissues from aborted fetuses in EMJH medium. CERRI et al. (1996), in Italy, isolated the serovar hardjo from kidneys of seropositive sheep by microscopic agglutination test. In this work, isolation was performed from animals without clinical signs of the disease (asymptomatic infection), which is important of the epidemiological point of view, as these animals may guarantee the persistence of the agent in the affected herds (FAINE et al., 1999).

In Brazil, there are few researches related to isolation of leptospires from naturally infected animals, as the majority of data is limited to serology (FREITAS et al., 2004). ADLER et al. (1986) and BOLIN et al. (1989) recognized the difficulty in isolating leptospires, despite its presence in samples. MOREIRA (1994), using 420 urine samples from bovines naturally infected, corresponding to 2,100 cultured tubes, obtained two leptospira isolations. The number of leptospira isolations obtained in this work is expressive when the total of cultured renal tissue samples (n = 160) was compared with that of the isolation samples (n = 4).

It was not possible to isolate leptospires from the subcultures that used contaminated tubes. ADLER et al. (1986) stated that the main problem in culturing leptospires is the contamination with other microorganisms, especially when attempting to culture from non-sterile sources such as urine and fetal tissues. The inclusion of antibiotics to inhibit the contamination in the culture medium has been recommended, but inhibitory substances have a detrimental effect on the multiplication phase of the leptospires (FAINE et al., 1999). THIERMANN (1984) stated that the most important factors for isolation of leptospires are aseptically collected material, quick processing, culture medium suitability and selective antibiotics.

The isolation of leptospires obtained in this study indicates that sheep slaughtered in the public slaughterhouse of Patos city may be sources of infection of the agent, exposing the slaughterhouse workers to occupational disease. The methodology used was shown to be efficient in the isolation of leptospires from sheep renal tissue and the identification of these isolates will allow for new epidemiological studies of leptospirosis.

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Received em 6/7/04
Accepted em 23/7/04