THE FIRST ISOLATION OF LEPTOSPIRA INTERROGANS SEROVAR POMONA FROM CATTLE IN BOTSWANA

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Serological evidence for the occurrence of L. pomona associated with abortions in cattle was supported by the successful isolation of the organism from the urine of a cow. Leptospirosis should be considered as a possible cause of abortion even in relatively dry regions.

Key words: Leptospira, cattle, abortion.

INTRODUCTION

Leptospiral abortions in cattle have recently been reported for the first time on the western borders of the Transvaal, in the Republic of South Africa. Following serological evidence for the involvement of Leptospira in abortions in cattle in the nearby Tuli Block in Botswana, a co-operative effort was instigated in an attempt to isolate the causative organism from the urine of animals involved.

MATERIALS AND METHODS

Animals and locality

The farm involved was a ranching enterprise with Brahman cross cattle situated on the Limpopo river on the Tropic of Capricorn in Botswana. Thirty-six adult cows which had aborted during the previous calving season were selected. A further 4 heifers were also used. Sporadic small abortion storms (4-5 abortions) had occurred on the property for a period of 6 years prior to this investigation.

Urine collection

Seven animals at a time were confined in a crush and given an intravenous injection, in the tail vein, of 500 mg of furosemide (Lasix, Hoechst). Mid-stream urine samples were then collected in sterile plastic beakers.

Media

Leptospira EMJH (Difco Laboratories) semi-solid [0,15 % Bacto agar (Difco Laboratories)] medium containing 0,5 mg 5-fluorouracil (Roche Products) per ml was used. This was dispensed in 5 ml quantities in screw-capped tissue culture tubes. A micropipette with sterile, disposable tips was used to inoculate 0,025 ml urine into 4 tubes from each animal. The media were kept at ambient temperature in the dark for 24 hours and then placed in an incubator at 29°C.

The media were examined twice weekly for 12 weeks for the appearance of a Dinger’s zone or for contamination. Contaminated specimens were examined under darkfield microscopy and discarded if no Leptospira were seen.

Urine samples were then collected in sterile plastic beakers. Medium showing a Dinger’s zone with pure culture Leptospira under darkfield examination were sub-cultured into fluid EMJH media. When good growth was seen on fluid media between 4-14 days the culture was titrated against standard Leptospira anti-sera for the serovars canicola, grippotyphosa, hardjo, hyos, icterohaemorragiæ, pomona and pyrogenes (Difco Laboratories).

Direct urine examination

After the inoculation of the media, 150 ml urine was preserved by adding 2,5 ml of a filtered formalin solution. This urine was later centrifuged at 1000 g for 10 min. The supernatant was decanted and re-centrifuged for 60 min at 3000 g. The pellets were examined under darkfield at 160x for the presence of typical Leptospira organisms. These examinations were carried out within 72 h of the urine collection.

Serology

Blood was collected from each animal by venipuncture of the tail vein using 10 ml vacuum tubes. The serum was subjected to the microscopic agglutination microvolume technique. The antigens used were 4-14 day cultures of L. canicola, L. grippotyphosa, L. hardjo (tarrassovi), L. icterohaemorragiæ, L. pomona and L. pyrogenes, maintained in the dark on liquid modified Stuart’s medium at 29°C. The end-point titre was taken as the dilution where 50 % of the organisms, as compared with the negative control, was either absent or visibly agglutinated and where a marked difference existed between it and the immediately preceding lower dilution. The reciprocal of the titre was used. A titre below 80 was regarded as negative, 80 as suspicious and anything above 80 as positive.

RESULTS

Serology

In 12 animals end-point titres of between 160 and 640 to L. hardjo were seen (Table 1). Eight animals were positive to L. pomona with titres of 160-2560 (Table 1). The highest titre to L. pomona was seen in Specimen no. 10. The 4 heifers included in the trials were serologically negative. Of the 36 cows which had aborted during the previous season only 9 were completely negative serologically. A further 3 had titres of 80 to L. hardjo while the rest had titres of 160 to L. hardjo and/or L. pomona.

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JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION – JUNE 1983

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0038-2809/83/02/0083-0084
Direct examination of formalinized urine specimens
Although leptospiral-like organisms were seen in 4 specimens under darkfield microscopy, their numbers were few and it was felt that they could not be positively identified beyond all doubt as Leptospira. Except for Specimen no. 10 there was no correlation between high-titrated sera and the presence of these organisms/artifacts.

Isolation and typing
A single successful isolate typed as L. pomona was made from Specimen no. 10.

DISCUSSION
The serological evidence indicted that L. pomona and/or L. hardjo could be involved as abortifacients. The fact that some of the cows were negative serologically and some had only low titres may be due to a number of factors. Infected animals do not always develop antibodies after infection. The time elapsed between the occurrence of the abortions and the attempt at isolation may have been sufficient for some animals to return to negative serological reactions. Not all the abortions need necessarily have been due to leptospirosis.

The fact that L. pomona was isolated from only one animal is disappointing, but once again a number of factors may have mitigated against more successful isolations. The time interval may be playing a role in that antibodies in the urine may have been present which could affect the viability of the organisms. Similarly the leptospiuric phase only lasts for a few months and may therefore have been intermittent at the time of the investigation. The failure to isolate L. hardjo could equally be due to the factors mentioned above or to the fact that L. hardjo is a much more fastidious organism than L. pomona.

The finding that a large percentage of the aborting cattle showed high titres to L. pomona and L. hardjo coupled with the isolation of L. pomona from one of them indicates that Leptospira could be considered as the causative organism in this case. As this is the first report of leptospirosis in cattle in Botswana it becomes imperative to consider the disease as a differential diagnosis especially where abortions are occurring and no aetiological diagnosis has been made. The association of leptospirosis with a wet environment may be the main reason for the failure in the past to consider it as a differential diagnosis in this relatively dry region.

ACKNOWLEDGEMENT
A special word of thanks is due to Miss Tertia Dreyer for her technical assistance in the isolation and typing of the organism.

REFERENCES