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LEPTOSPIRA INTERROGANS SEROVAR *HARDJO* INFECTION IN MALAYSIAN INDIGENOUS CATTLE

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SUMMARY

Six healthy Kedah-Kelantan calves of approximately 8 months old were selected for this study. Four calves were exposed to serovar *hardjo* by either the conjunctival or intravenous route whilst another two calves were housed together with the four infected calves to study the transmission of *hardjo* infection. Clinical signs of *hardjo* infection were not observed. Leptospiremia was first detected on post-inoculation day (PID) 7 and lasted until PID 13 in the infected cattle. However, it was found that dark-field microscopy was not sensitive enough to detect leptospires in the urine and blood samples. Leptospiruria was only present in animals that have been challenged through the conjunctival route. It was first detected on PID 50 and persisted to PID 148. Leptospires were not isolated from the kidneys of the infected cattle. *Leptospira interrogans* serovar *hardjo* appeared to stimulate low antibody response in one of the animals infected conjunctivally (titre of 1:320). There was no evidence of natural transmission of *hardjo* infection to the in-contact animals even tough they were housed together with the infected animals.

Keywords : *Leptospira interrogans* serovar *hardjo*, cattle.

INTRODUCTION

Leptospira interrogans serovar *hardjo* is one of the important causes of abortion and infertility in cattle worldwide (Ellis *et al.*, 1976). It was found to be endemic in cattle in Malaysia (Bahaman *et al.*, 1987). Studies have indicated that cattle are the maintenance host for serovar *hardjo,* shedding leptospires through urine for long periods of time. The duration of leptospiral excretion varies while leptospiruria may continue for several weeks or persist for more than a year (Mackintosh *et al.*, 1980; Thiermann, 1982). In Malaysia, serovar *hardjo* usually causes subclinical infection in cattle.

This study investigates the infectivity of serovar *hardjo* in Malaysian indigenous cattle and determines the role of infected cattle as maintenance host for serovar *hardjo* infection.

MATERIALS AND METHODS

Experimental animals

Six healthy 8-month old Kedah-Kelantan calves were selected for the study. They were free of detectable leptospiral antibodies based on the microscopic agglutination test (MAT) and the enzyme-linked immunosorbent assay (ELISA). The calves were kept in isolation for two months before being exposed to serovar *hardjo*.

Inoculum

A local serovar *hardjo* isolate, obtained from bovine urine, was passaged through a hamster and re-isolated from its kidneys. The culture was grown for seven days at 30°C in liquid Johnson and Seiter (JS) medium containing 10% rabbit serum and prepared as inoculum to infect the experimental calves.

Experimental procedure

Two calves were infected with 1mL inoculum containing $8x10^6$ leptospires per mL of liquid culture intravenously through the jugular vein. Another two calves were infected using the same inoculum through the conjunctival space. Following the infections, two uninfected calves were housed together with the infected calves to study the natural transmission. Clinical signs were monitored and rectal temperature was recorded before and after the infections. Each animal was observed for clinical signs twice daily for the first 14 days post-inoculation.

Blood samples for culture and serological examinations were collected at two months and one week prior to the inoculation, on the day of inoculation and daily post-inoculation for a period of 14 days. Samplings were then carried out weekly until post-inoculation day (PID) 365. Urine samples for culture and direct dark-field examination were collected once before inoculation and then weekly until PID 365. At the end of the experiment (PID 365) calves were slaughtered and kidneys were collected for leptospiral isolation.

Direct examination of blood and urine samples by dark-field microscopy

Blood and urine samples were examined for the presence of leptospires by dark-field microscopy. Fifty mL of urine sample were centrifuged at 8000 rpm for

10 min before the pellet was dissolved in 100μL liquid JS medium. A drop of the sample was then examined under dark-field microscope. The blood samples were pipetted into micro-hematocrit tubes and centrifuged at 10,000 rpm for 10 min. The buffy coat was collected and examined under dark-field microscope for the presence of leptospires.

Bacteria culture

Blood samples were cultured immediately after collection. Two drops of blood were inoculated directly into two bottles of semisolid (0.17% agar) JS medium while another two drops were inoculated into another two bottles of semisolid medium containing 200 μL/mL of 5-fluorouracil (5FU). Mid stream urine samples were diluted 1:10 in JS basal medium before two drops of undiluted urine and two drops of diluted urine samples were inoculated into semisolid JS medium containing 200 μL/mL of 5FU. An additional series of medium containing 400 μL/mL of 5FU were also inoculated. Immediately after slaughter, the kidneys were aseptically removed from their capsule before 25 gm were placed in 225 mL JS medium and homogenised for five min in a stomacher. The homogenate was centrifuged at 3500 rpm for five min to deposit larger tissue fragments. The supernatant was inoculated into four bottles of semisolid JS medium containing 200 μL/mL of 5FU and another four bottles containing 400 μL/mL of 5FU. Cultures were incubated at 30°C for 12 weeks and examined by dark-field microscopy at two-weekly intervals.

Serological examination

Serum samples were tested against live field *hardjo* isolate by the microscopic agglutination test (MAT) as described by Cole *et al.* (1973). The minimum serum dilution performed was 1:20.

RESULTS

Clinical signs

The infected animals appeared clinically normal throughout the experimental period of 365 days. Clinical signs of leptospiral infection were not observed and no significant temperature responses were detected during the infection.

Leptospiral isolation

All urine and blood samples taken throughout the experimental period were negative for leptospires on direct dark-field microscopy examination. However, leptospires were successfully cultured from blood of the two calves that were challenged intravenously and from the other two calves that were exposed by the conjunctival route. There was no leptospiral isolation from the blood samples of the two in-contact calves. Positive blood cultures were first obtained on PID 7 and continued until PID 13.

Leptospiruria was detected only in the two animals exposed by the conjunctival route. It was first detected on PID 50 and PID 106 respectively and lasted until PID 148. All isolates were serologically identified as serovar *hardjo* by MAT. However, leptospires were not isolated from the kidneys of any of the experimental animals.

Serological findings

Generally, the infection was detected by MAT in all inoculated calves. However, the in-contact calves remained serologically negative to *hardjo* throughout the experiment. Leptospiral agglutinating titres were detected in the serum of the intravenously infected calves as early as PID 7 (titre 1:40) and increased to 1:320 with time. The period of titres detection lasted between PID 14 to PID 35. One of the conjunctivally exposed calves was sero-positive by MAT with titre 1:160 until PID 56. The titre (1:20) for the second calf was first detected on PID 49 and rapidly increased to 1:160 at PID 7 before decreased to 1:20 at PID 301.

DISCUSSION

It was observed in the present experiment that there were no signs of *hardjo* infection such as elevated body temperature and anorexia. All urine and blood samples directly examined by dark-field microscopy were negative for leptospires throughout the experiment. In New Zealand, Mackintosh *et al.* (1981) also failed to detect leptospiruria in cattle by dark-field microscopy. The fact that no urine and blood samples were positive by dark-field microscopy indicated that the number of leptospires in the samples was at a low level. In an earlier study by Turner (1970), it was shown that the number of leptospires present in the urine was usually less than 10^4 leptospires/mL. It is possible, however, that dark-field microscopy may not be sensitive enough to detect small numbers of leptospires in the urine and blood samples. The successful detection of leptospires by culture from blood and urine samples in this study supported previous observation that urine and blood culture is more sensitive than direct dark-field microscopic examination (Ris and Hamel, 1978). This study also found that leptospiremia occurred as early as PID 7 and lasted until PID 13 in all infected animals.

Leptospiruria was only detected in animals challenged by the conjunctival route. Leptospira was first detected in the urine as early as PID 50. Thiermann and Handsaker (1985) reported that leptospiruria was first detected in cattle inoculated intravenously as early as PID 14. Failure to isolate leptospires in early stage of infection was probably due

to the low number of leptospires present in the urine samples. Leptospiruria persisted for a period between 113 to 148 days. Amatredjo and Campbell (1975) concluded that leptospiruria usually persists for a period between 26 to 118 days in cattle. However, shedding of serovar *hardjo* by cattle for a period of more than a year has been reported in New Zealand (Mackintosh *et al.*, 1980). Hodges and Ris (1974) reported that urinary shedding of serovar *hardjo* was first observed 26 to 28 days after inoculation and persisted until the day 42 to 54. The shedding duration reported here was longer than that reported by Hodges and Ris (1974). Although leptospira was isolated from urine of cattle infected conjunctivally, no isolation was following intravenous expose. This, however, is in contrast to an earlier study in which serovar *hardjo* was infected conjunctivally and intravenously but failed to re-isolate leptospira from urine and kidneys (Thiermann and Handsaker, 1985).

The two in-contact calves remained serologically and culturally negative throughout the experiment, even though they would have been exposed to the leptospiruric calves. Mackintosh *et al.* (1981) had similarly reported that in-contact calves that have been exposed to the leptospiruric calves remained serologically and culturally negative to leptospiral infection. Earlier, Mackintosh *et al.* (1980) found that there was a rapid spread of serovar *hardjo* infection in calves under similar conditions. This lack of transmission from calves to calves indicated that these animals were unlikely to act as maintenance host for serovar *hardjo*. Disability of serovar *hardjo* to transmit infection may also due to the resistant local breed used in this study. The Kedah-Kelantan breed, which is indigenous to Malaysia, is noted for resistant to many infectious diseases. Blackmore and Hathaway (1979), however, concluded that New Zealand cattle are the maintenance host for serovar *hardjo*.

Leptospira interrogans serovar *hardjo* appears to produce only a minimal antibody response in experimental cattle. A titre of 1:320 was the highest recorded in the present study. Intravenously infected cattle produced early MAT responses (PID 7) and persisted to PID 35. Thiermann and Handsaker (1985) have reported similar findings, in which intravenously infected animals showed early MAT responses (PID 7) and remained sero-positive for up to 50 to 60 days. The conjunctivally exposed calves showed slower respond (PID 49) but persisted longer (up to PID 301). The results of this study indicated that conjunctival route of infection with $8x10^6$ leptospires/mL was a more natural and successful route to infect cattle with serovar *hardjo*.

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RINGKASAN

JANGKITAN LEPTOPSPIRA INTERROGANS *SEROVAR* HARDJO *PADA LEMBU ASLI MALAYSIA*

Enam ekor anak lembu Kedah-Kelantan yang berumur 8 bulan telah digunakan dimana empat ekor telah disuntik dengan serovar hardjo samada secara konjuktiva atau intravena. Dua ekor lagi dipelihara bersama empat ekor yang terjangkit untuk mengkaji kaedah pemindahan jangkitan hardjo. Walaupun leptospiremia dikesan pada hari ketujuh pasca penginokultan (PID) dan berakhir pada hari ketiga belas, tiada petanda klinikal leptospirosis dilihat. Adalah juga didapati bahawa mikroskopi lapangan gelap tidak begitu peka untuk mengesan leptospira dalam sampel urin dan darah. Leptospiruria hanya ujud pada haiwan yang disuntik secara konjuktiva. Ia mula dikesan pada PID 50 dan dan kekal sehingga PID 148. Tiada isolat leptospira diperolehi daripada ginjal haiwan yang terjangkit. Leptospira interrogans serovar hardjo dilihat mengaruh gerakbalas antibodi yang rendah pada haiwan yang terjangkit secara konjuktiva (titer 1:320). Pemindahan semulajadi jangkitan hardjo secara sentuhan dengan haiwan terjangkit tidak terbukti.