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LEPTOSPIRA INTERROGANS SEROVAR *HARDJO* INFECTION IN CATTLE IN MALAYSIA: SEROLOGICAL AND BACTERIOLOGICAL PREVALENCE

S. Khairani-Bejo and A. R. Bahaman

Institute of Bioscience
Universiti Putra Malaysia
43400, Serdang, Selangor, Malaysia

SUMMARY

Serological study revealed a high prevalence rate (36%) of leptospiral infection in the cattle examined. Antibodies against five leptospiral serovars namely *australis, canicola, hardjo, javanica* and *pomona* were detected by MAT. The main serovar affecting the herd was shown to be serovar *hardjo* with 19% serological prevalence. The study showed similar sensitivity and specificity for both ELISA and MAT (70.8% Kappa test). Bacteriological study showed that 1.2% (3/244) of the animals had leptospiral infection. The 3 leptospiral isolates were identified as serovar *hardjo* (2) and *L. biflexa* (1). The results of this study support early reports that cattle are the maintenance host of serovar *hardjo* in this country.

Keywords : *Leptospira interrogans* serovar *hardjo*, cattle, West Malaysia

INTRODUCTION

Leptospira interrogans serovar *hardjo* infection has been reported to be endemic in cattle population in West Malaysia (Bahaman *et al.*, 1987) and in many other cattle rearing countries (Ellis *et al.*, 1981). Infection by serovar *hardjo* in cattle can cause economic losses due to decreased milk production, infertility, stillbirth, abortion, mastitis and occasionally death. Apart from the economic importance, serovar *hardjo* infection is an important zoonotic disease. Cattle infected with serovar *hardjo* were often chronic renal carriers. Epidemiological studies have indicated that cattle are an important maintenance host for serovar *hardjo* and the main source of human infection (Ellis *et al.*, 1981). Bahaman *et al.* (1987) studied the prevalence of leptospiral infection in domestic animals in West Malaysia and found that cattle had the highest prevalence (40.5%) with 34% were due to serovar *hardjo*. The high serological prevalence of leptospiral infection reported by previous workers (Arunasalam 1975; Leong and Maamor 1975; Bahaman *et al.*, 1987), indicated that cattle in West Malaysia are the maintenance host for serovar *hardjo*.

The microscopic agglutination test (MAT) is the standard serological test for diagnosis of serovar *hardjo* infection in the field, but it is more reliable on a herd basis (Hathaway *et al.*, 1986). Other disadvantages of the test include the need to maintain live antigen, which is a health hazard and the subjectivity in the reading of the results. To overcome these difficulties, enzyme-linked immunosorbent assay (ELISA) has been developed which measures different level of immunoglobulins specific to serovar *hardjo* (Bercovich *et al.*, 1990). However the definitive diagnosis of leptospiral infection is usually achieved by isolating and identifying of the infecting organism (Smith *et al.*, 1984).

The objectives of this study were to determine the serological and bacteriological prevalence of serovar *hardjo* infection in cattle and determine the sensitivity and specificity of MAT and ELISA in the diagnosis of serovar *hardjo* infection in cattle.

MATERIALS AND METHODS

Urine and serum samples

Two hundreds and forty four (244) urine and 318 serum samples were collected from yearling cattle in selected farms in West Malaysia. Mid-stream urine samples were collected into clean sterile universal bottles. Blood samples were collected by venopuncture of the coccygeal vein using 18G needle and plain venoject tubes. The blood samples were left to clot in ice flasks and stored at -20°C until used for serological study.

Serology

Serum samples were examined for presence of leptospiral antibodies by MAT and ELISA. The MAT technique was as described by Bercovich *et al.* (1990). Two-fold serial dilution of sera, starting with 1:25, were made in sterile phosphate buffer saline (PBS). Equal volume of live antigen of serovars *hardjo, pomona, canicola, australis* and *icterohaemorrhagiae* were added to each dilution and incubated at 37°C for 90 min. A serum was considered to be positive when approximately 50% or more of the leptopsires were agglutinated.

The ELISA procedure used in this study was as described by Bercovich *et al.* (1990). The protein antigen was the outer envelope of the leptospires, lysed by boiling with sodium dodecyl sulphate (SDS) at 97°C for five min (Bey *et al.*, 1974). The lysed leptospires was then centrifuged at 12,000 rpm for 10 min and the supernatant was then used as antigen. The microtitre plate (Flow Laboratories, Inc.) was coated with 5μg protein per well diluted in 0.05M carbonate/ bicarbonate buffer (pH 9.6). Diluted serum samples (1:200) were then added, followed by goat-anti-cattle antiserum conjugated with horseradish peroxidase and finally the 0.4 mM of ABTS peroxidase substrate (2,2'-azino-di [3-ethylbenzthiazoline sulfonate 6]) and 2mM hydrogen peroxide in 0.05M citrate buffer (pH 4.0) as detecting system. Sera were recorded as positive if their OD reading was two times higher than the mean OD reading of the negative control sera.

Isolation and identification of leptospires

A drop of undiluted and a 10-fold dilution of urine samples were immediately inoculated into semi-solid Johnson and Seiter (JS) medium containing 200 μg/mL of 5-fluorouracil (5-FU) to isolate the leptospires. All cultures were incubated at 30°C, examined at 5 to 7 days interval for presence of leptospires and discarded as negative after 12 weeks of incubation. The isolates were identified by their typical morphology, motility and growth inhibition at 13°C in 8-Azaguanine. Pathogenicity test was performed by inoculating leptospiral isolates through intraperitoneal route into weanling hamster (*Mesocricetus auratus*) followed by observation of clinical signs and mortality. The isolates were identified to serogroup level by microscopic agglutination test (MAT) against 16 rabbit hyperimmune sera representing the 16 important leptospiral serogroup.

RESULTS

Out of 318 cattle sera tested, 114 (36%) were found positive for leptospiral infection. Antibodies to five serovars were detected, namely *australis, canicola, hardjo, javanica* and *pomona*. The main serovar affecting the herd was shown to be serovar *hardjo* with 19% serological prevalence. One hundred and seventeen (37%) sera examined using ELISA were positive to leptospiral infection. The study showed that overall agreement between ELISA and MAT is 70.8% (Kappa test).

Three urine samples were positive for leptospires on culture. Isolate SS1946 was seen in the urine culture on the third week of incubation whilst isolates H41 and F33 were seen on the sixth week of incubation. The isolate SS1946 was able to grow at 13°C whilst the isolate H41 and F33 were not. The growth of isolate H41 and F33 was completely inhibited by 8-Azaguanine after the second passage but not for isolate SS1946. This indicated that isolate SS1946 is a saprophytic leptospira.

All isolates failed to produce mortality to the weanling hamsters. Isolate SS1946 did not respond to any of the hyperimmune sera tested whilst isolates H41 and F33 reacted to serovar *hardjo*.

DISCUSSION

All animals in the study herds appeared healthy and there was no indication of any clinical sign suggestive of leptospirosis. This indicated that natural leptospiral infection in cattle in West Malaysia is inapparent. The lack of clinical signs and the high sero-prevalence suggested that the infection is endemic in the herds.

The results of this study showed that ELISA was more sensitive for the detection of leptospiral antibodies in bovine serum. Since the boiled antigens used for ELISA were genus-specific, more positive reactions were expected. The MAT gave a slightly lower prevalence of leptospiral infection since the reaction was mostly based on agglutination of surface antigens present on live organisms (Thierman and Garrett, 1983). The simple and safe procedure of ELISA as compared to MAT will be an advantage in screening serum samples for the presence of leptospiral infection caused by numerous leptospiral serovars. These findings disclosed that the ELISA was a useful epidemiological tool.

Antibodies against five leptospiral serovars were detected by MAT namely *australis, canicola, hardjo, javanica* and *pomona*. Antibody to serovar *hardjo* was the main (19%) serovar disclosed. Definitive diagnosis of leptospirosis is usually by isolating the infecting organism (Thiermann, 1984). Urine samples were obtained from yearling cattle for this purpose as it is established that leptospires persist in kidneys and are excreted with urine for long periods (White *et al.*, 1982). They are able to persist and multiply in the renal tubules as they are protected from circulating antibodies and phagocytes (Smith *et al.*, 1994). Leptospires are then excreted, often intermittently, in urine for a variable period. It is generally assumed that shedding of leptospires in the urine starts in the second week of illness and may last for several months (Michna, 1970). Three urine samples were positive to leptospires on culture. Positive culture (isolate SS1946) was detected on the third week of incubation. However, positive cultures of isolates H41 and F33 were detected only on the sixth week of incubation. Microscopic agglutination test (MAT) showed that isolate SS1946 did not react to any of the hyperimmune

sera tested whilst isolates H41 and F33 reacted to *hardjo*. WHO Collaborating Centre for Reference and Research on Leptospirosis, Brisbane, Australia confirmed that isolate SS1946 was *L. biflexa* whilst both isolates H41 and F33 were serovar *hardjo*.

Antibodies to serovar *hardjo* were the most prevalent (19%) and this serovar was also isolated from the urine samples of two cattle in the studied farms. However, all the cattle in the farms appeared to be healthy and there were no clinical sign to leptospirosis. Animals are considered to be a maintenance host when they are highly susceptible to infection but the pathogenicity of the serovar is low for the host, long term kidney infection or naturally transmitting infection within the host species (Blackmore and Hathaway, 1980). The findings from this present study agree with the factors as described by Blackmore and Hathaway (1980) and concluded that cattle are the maintenance host of serovar *hardjo* infection in West Malaysia.

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RINGKASAN

JANGKITAN LEPTOSPIRA INTERROGANS *SEROVAR* HARDJO *PADA LEMBU DI MALAYSIA: PREVALENS SEROLOGI DAN BAKTERIOLOGI*

Kadar prevalens yang tinggi (36%) bagi jangkitan leptospira terhadap lima serovar telah dikesan melalui keadah MAT pada lembu.Serovar utama yang menjangkiti kelompok adalah serovar pomona (19%). Kaijan menunjukkan kepekaan dan ketepatan serupa bagi ELISA dan MAT (70.8% ujian Kappa). Kajian bakteriologi menunjukkan bahawa 1.2% (3/244) daripada haiwan mempunyai jangkitan leptospira. Isolat leptospira dikenalpasti sebagai serovar hardjo (2) dan biflexa (1). Keputusan kajian ini menyokong bahwa lembu merupakan hos senggaraan serovar hardjo di negeri ini.