Comparison between ELISA and the Microscopic Agglutination Test for the Diagnosis of Bovine Leptospirosis

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Keywords

Cattle – Leptospirosis – Diagnosis – ELISA.

Summary

An indirect enzyme-linked immunosorbent assay (ELISA) was compared with the microscopic agglutination test (MAT) for the diagnosis of bovine leptospirosis. A total of 170 random bovine serum samples were collected from cattle farms in Malaysia. The serum samples were examined for the presence of antibodies to leptospires using ELISA and MAT. Ninety-six (56.5%) sera were positive to ELISA, while 47 (27.7%) were positive to MAT. All samples positive to MAT were positive to ELISA, while there was no sample positive to MAT and negative to ELISA. ELISA showed 100% sensitivity compared to MAT. ELISA appears as a better alternative to MAT for the diagnosis of bovine leptospirosis.

■ INTRODUCTION

Leptospirosis is a serious zoonotic disease with important veterinary and public health impacts (7). The disease is caused by *Leptospira interrogans*. It causes important economic losses in livestock. Although it is usually mild and often subclinical, it can lead to great losses due to abortion, stillbirth, infertility, mastitis, weak progeny, decreased milk production and, with certain leptospiral serovars, death (4, 13).

Diagnosis of leptospirosis based on serology is the most suitable for the rapid testing of a large number of individuals (5, 7, 11). Serodiagnostic methods used for leptospirosis included the microscopic agglutination test (MAT), hemagglutination (HA) test, fluorescent antibody (FA) and complement fixation test (CFT). Drawbacks of most of these serological tests led to the rapid development of the enzyme-linked immunosorbent assay (ELISA) in the diagnosis of bovine leptospirosis. Different types of ELISA have been developed and were found to be useful for the diagnosis of leptospirosis (1, 10, 12, 14). The main objective of this study was to compare ELISA and MAT as screening tests for diagnosis of bovine leptospirosis.

■ MATERIALS AND METHODS

Test sera

One hundred and seventy serum samples were collected from two dairy farms in Malaysia: 70 from the University Putra Malaysia Farm and 100 from the Malaysian Agriculture Research and Development Institute (Bahang) Farm. The samples were random and the animals examined were of both sexes with age range from six months to four years. All serum samples were examined for antibodies to *Leptospira* by ELISA and MAT.

ELISA

The antigen used with ELISA was a genus-specific antigen (lipopolysaccaride) derived from boiling leptospiral culture (serovar *hardjo*). Goat antibovine IgG labeled with horseradish peroxidase was used as conjugate. An indirect ELISA technique was used based on protocols by El Jalii et al. (6). It was carried out with a

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working volume of 100 µl of each reagent. The 96 wells of microtitration plates (Dynex, Immulon, USA) were coated with 100 µl of 1/1600 *hardjo* LPS antigen in 0.05 M carbonate/bicarbonate buffer pH 9.6, incubated at 4°C overnight, and washed four times with washing buffer containing 0.05% (v/v) Tween 20 in PBS pH 7.0 using a microplate washer (Nunk Immuno Washer 8, Denmark). Then, 100 µl of 2% BSA fraction V (Sigma, UK) were added to the wells to prevent non-specific binding and incubated for 2 h at 37°C. After washing four times, 100 µl of known bovine positive and negative sera (1/200 diluted sera in PBS pH 7.2 containing 0.03% Tween 20) as controls and the test sera were added to wells in duplicate and incubated at 37°C for one hour. The plate was then washed four times and 100 µl of pre-diluted goat anti-bovine peroxide conjugated immunoglobulin (IgG) (KPL, USA) in PBS-Tween 20 were added and incubated at 37°C for one hour, washed four times, and 100 µl of 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 2 mM hydrogen peroxide in 0.05 M citrate buffer pH 4.0 substrate were added to each well and incubated at room temperature in the dark for 10 min. The results were read with the aid of a spectrophotometer (Titretek Multiskan Plus, Finland) using 405 nanometers wavelength. Sera were reported as positive if their optical density (OD) readings were higher than the OD readings of the mean negative controls plus three standard deviations.

MAT

The antigens used with MAT were made up of 4-8 day-old live cultures of the following serovars: *hardjo, canicola, icterohemorrhagiae, australis, pomona* and *patoc* strain *patoc1*. MAT was performed as described by Cole et al. (5) with some modifications. Each serum sample was initially diluted (1/25) with phosphate buffer saline (PBS) pH 7.2 in a microtitration plate (Greiner Labrotechnik), 25 µl of PBS were placed into each well of the plate and an equal volume of the diluted serum sample was placed in the first row (A row) of the plate. The diluted serum which was then 1/50 was serially diluted (two-fold) from A row to H row using a hand-held 0.025 ml microdiluter. Then, 25 µl of the live antigens (4-8 day-old cultures containing 10^8 leptospires/ml) were added to each well. Thus, each well contained an equal volume of the diluted serum sample and the antigen. For each serum sample tested, there were eight dilutions ranging from 1/100 to 1/12,800. The plate was gently shaken for 15-20 s to mix the contents, covered to exclude debris and prevent evaporation, and incubated for one hour at 37°C. The test was read by transferring a drop from each well onto a glass microscope slide. The drops were examined by dark-field microscopy (x200). A positive reaction was regarded as one in which 50% or more of the antigen (live leptospires) were agglutinated. The titer end point was taken as the last well in which 50% or more agglutination was observed.

Statistical analysis

Stata 98/NT was used for analysis.

■ RESULTS

Out of the 170 serum samples examined by MAT, 47 (27.7%) were positive to MAT, while 123 (72.3%) were negative. Forty (85.1%) of MAT positive animals were found to be infected by serovar *hardjo,* which is a predominant infecting serovar. Five (10.6%) were infected by *pomona,* and two (4.3%) by *australis*.

Out of the 170 serum samples examined by ELISA, 96 (56.5%) were positive, while 74 (43.5%) were negative.

When comparing MAT and ELISA, 96 (56.5%) were positive to ELISA, while 47 (56.5%) were positive to MAT.

All samples which were positive to MAT were positive to ELISA.

There was no sample positive to MAT and negative to ELISA. Forty-nine (28.8%) were positive to ELISA and negative to MAT.

■ DISCUSSION

Leptospirosis is one of the diseases with economic impact, so its diagnosis and serosurveillance are very important for any control program. Many tests have been used in the field as screening tests of leptospirosis. The present study was designed to compare ELISA and MAT for screening of bovine leptospirosis. Serological evidence of three leptospiral serovars was revealed from animal sera tested, i.e. *hardjo, pomona* and *australis,* with serovar *hardjo* as the most prevalent (85.1% prevalence). In Malaysia, 38 serovars from 13 serogroups have been known to occur (2). The high prevalence of *Leptospira* serovar *hardjo* infection in this study was significant because it supported previous studies on animal leptospirosis in Malaysia which reported that cattle maintained *Leptospira* serovar *hardjo* (3, 8).

Using MAT, three leptospiral antibodies were revealed in this study. ELISA detected positive samples to leptospirosis from sera infected with these three serovars. The antigen used in this study was heat-extracted and was prepared from a single serovar (*hardjo*). It permitted the detection of infection caused by different serovars. The use of a single serovar to prepare the antigen for ELISA appeared reliable and easy. This antigen was stable and could be stored for a long time either in liquid state or coated onto polystyrene plates. On the other hand, MAT used live leptospires belonging to different serovars, which had to be propagated and subcultured continuously to carry out the test. This was very tedious and created a risk of infection to laboratory personnel. ELISA showed 100% sensitivity compared to MAT. No sample positive to MAT was negative to ELISA. This indicated that ELISA was more sensitive than MAT and offered many advantages over MAT. The technique is semi-automated, easy and reproducible. ELISA can potentially be used as a screening test for leptospirosis (9, 15). In conclusion, ELISA was sensitive and could detect antibodies to multiple pathogenic *Leptospira* serovars. It is a good assay for bovine leptospirosis screening.

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Résumé

El Jalii I.M. Comparaison entre le test Elisa et le test de microagglutination dans le diagnostic de la leptospirose bovine

Un test Elisa indirect a été comparé au test de microaaglutination (MAT) dans le diagnostic de la leptospirose bovine. Au total 170 échantillons sériques aléatoires on été prélevés sur des bovins dans des fermes en Malaisie. L'examen des échantillons a porté sur la recherche d'anticorps contre *Leptospira* en utilisant Elisa et MAT. Quatre-vingt-seize (56,5 p. 100) échantillons ont été positifs à l'Elisa, alors que 47 (27,7 p. 100) l'ont été au MAT. Tous les échantillons qui ont été positifs au MAT l'ont aussi été à l'Elisa ; il n'y a pas eu d'échantillon positif au MAT et négatif à l'Elisa. La sensibilité d'Elisa a été de 100 p. 100 comparée à celle de MAT. Elisa apparaît comme étant la meilleure technique des deux dans le diagnostic de la leptospirose bovine.

Mots-clés : Bovin – Leptospirose – Diagnostic – Test Elisa.

Resumen

El Jalii I.M. Comparación entre el ensayo de inmunoabsorbente ligado a enzimas y el test de microaglutinación para el diagnóstico de la leptospirosis bovina

Se comparó un ensayo inmunoabsorbente ligado a enzimas (ELISA) indirecto con el test de microaglutinacion (MAT) para el diagnóstico de la leptospirosis bovina. Se colectó al azar un total de 170 sueros bovinos proveniente de ganado de fincas en Malasia. Las muestras de suero se examinaron para la presencia de anticuerpos de leptospirosis usando ELISA y MAT. Noventa y seis (56,5%) de los sueros fueron positivos con ELISA, mientras que 47 (27,7%) fueron positivos con MAT. Todas las muestras positivas mediante MAT fueron positivas mediante ELISA, mientras que ninguna muestra fue positiva mediante MAT y negativa mediante ELISA. ELISA mostró 100% de sensitividad comparado con el MAT. ELISA parece ser una mejor alternativa que MAT para el diagnóstico de la leptospirosis bovina.

Palabras clave: Ganado bovino – Leptospirosis – Diagnóstico – ELISA.