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EPIDEMIOLOGY OF LEPTOSPIRAINTERROGANS SEROVAR HARDJO INFECTION IN CATTLE

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By

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The serological prevalence of *Leptospira interrogans* serovar *hardjo* (hereafter referred to serovar *hardjo*) infection in cattle in this present study was 30%. Water samples from stagnant water, pond water, tank water and drain water collected from the farms were positive to *Leptospira biflexa* (40%). Twenty-four per cent of soil samples obtained from three different types of soil namely clay, loam and sand in the farms were also positive to *Leptospira biflexa*. However, serovar *hardjo* or other pathogenic leptospires were not isolated in the urine, soil and water samples collected in the farms. Clinical sign of leptospiral infection was not observed in the cattle on the farms. The leptospiral isolates were further characterized using bacterial restriction endonuclease DNA analysis (BRENDA), polyacrylamide gel electrophoresis (PAGE) and Western blotting. It was confirmed that the leptospiral isolates did not belong to serovar *hardjo*.

Under experimental condition it was demonstrated that cattle are able to maintain serovar *hardjo*. Six female 8-months-old Kedah-Kelantan calves were used in this trial. Leptospiremia occurred as early as 7 days post-inoculation and lasted for 13 days following intra-conjunctival inoculation. Antibody against serovar *hardjo* was first detected at day 7 post inoculation, then raised to a high level at day 14 post-inoculation and maintained at the same level up to 365 days post inoculation. Leptospiruria was first detected on day 49 post inoculation and maintained up to day 147 post-inoculation. Histologically serovar *hardjo* was detected in the renal tubule at the end of the trial using immunoperoxidase staining. No clinical signs of leptospiral infection was observed in the same animals throughout the trial. Identification of the leptospiral isolates obtained from the inoculum and urine samples of the experimental animals using bacterial restriction endonuclease DNA analysis (BRENDA) and polymerase chain reaction (PCR) showed that both isolates were serovar *hardjo*.

The study showed that serovar *hardjo* can survive in rain water up to 264 hours (11 days) under experimental condition. *Leptospira interrogans* serovar *hardjo* can survive up to 72 hours (3days) in diluted urine in Malaysian field condition and up to 984 hours (41 days) at 4°C. *Leptospira interrogans* serovar *hardjo* can survive in chlorinated drinking water up to 120 hours (5 days) but was killed immediately in seawater. The organism can survive in soil samples

up to 144 hours (6 days). The contaminated environment with serovar *hardjo* can transmit infection of the organism to susceptible animals.

It is evident that serovar *hardjo* infection is present in cattle farms in Malaysia. Cattle in Malaysia have a potential of maintaining serovar *hardjo*. *Leptospira interrogans* serovar *hardjo* has been shown to survive in water and soil for a long time in Malaysian field condition and the organisms can be transmitted to susceptible animals.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan ijazah Doktor Falsafah

EPIDEMIOLOGI JANGKITAN *LEPTOSPIRA INTERROGANS* SEROVAR *HARDJO* PADA LEMBU

Oleh:

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Prevalan jangkitan *Leptospira interrogans* serovar *hardjo* (selepas daripada ini dipanggil sebagai serovar *hardjo*) pada lembu dalam kajian serologi adalah 30%. Sampel air bertakung, air kolam, air tangki dan air parit yang diambil dari ladang lembu adalah positif kepada *Leptospira biflexa* (40%). Tiga jenis tanah yang berbeza seperti tanah liat, tanah peroi dan tanah pasir telah di ambil dari ladang tersebut dan didapati 24% sampel tanah juga positif kepada *Leptospira biflexa.* Walaubagaimanapun *Leptospira interrogan* serovar *hardjo* dan lain-lain leptospira patogenik tidak dapat diasingkan dari sampel air kencing lembu, tanah dan air yang diambil dari ladang tersebut. Kesemua lembu di ladang kajian tidak ada menunjukkan tanda-tanda klinikal jangkitan *Leptospira.* Isolat *Leptospira* seterusnya dicirikan menggunakan analisa DNA bakteria endonuklias penyekat, elektroporesis gel poliakrilamid dan pemblotan Western. Isolat *Leptospira* yang diasingkan dalam kajian ini adalah pasti bukan serovar *hardjo*.

Dalam keadaan eksperimen menunjukkan lembu dapat penyaraan serovar *hardjo*. Enam ekor lembu betina baka Kedah-Kelantan berumur lapan bulan telah digunakan dalam percubaan ini. Leptospiremia berlaku seawal tujuh hari selepas inokulasi dan berakhir sehingga hari ke-13 berikutan inokulasi ke dalam konjunktiva. Antibodi terhadap serovar *hardjo* telah dikesan pertama kalinya pada hari ke tujuh selepas inokulasi, kemudian meningkat ke tahap yang tinggi pada hari ke-14 selepas inokulasi dan seterusnya kekal pada tahap yang sama sehingga hari ke-365 selepas inokulasi. Leptospiruria telah dikesan pertama kalinya pada hari ke-49 selepas inokulasi dan kekal sehingga hari ke-147 selepas inokulasi. Kajian histologi menggunakan pewarnaan immunoperoksides mengesan serovar *hardjo* di tubul ginjal pada akhir percubaan. Tanda klinikal jangkitan leptospira tidak ditunjukkan oleh kesemua lembu sepanjang percubaan ini. Pengenalpastian menggunakan kaedah analisa DNA bakteria endonuklias penyekat dan tindakbalas berangkai polimerase menunjukkan kedua-dua isolat dari lembu ujikaji dan inokulum adalah dikenalpasti sebagai serovar *hardjo*.

Dalam keadaan eksperimen serovar *hardjo* boleh terus hidup sehingga 264 jam (11 hari) didalam air hujan. Dalam cuaca persekitaran Malaysia serovar *hardjo* boleh terus hidup sehingga 72 jam (3 hari) di dalam air kencing yang dicairkan. Pada suhu 4°C serovar *hardjo* terus hidup sehingga 984 jam (41 days).

Leptospira interrogans serovar *hardjo* boleh terus hidup dalam air paip yang berklorin sehingga 120 jam (5 hari) tetapi mati serta merta di dalam air laut. Organisma ini dapat terus hidup dalam tanah sehingga 144 jam (6 hari). Alam sekitar yang telah dicemari oleh serovar *hardjo* dapat menyebarkan jangkitan oleh organisma ini kepada haiwan yang mudah terkena jangkitan.

Kesimpulannya, jangkitan serovar *hardjo* adalah terdapat di ladang lembu di Malaysia. Lembu di Malaysia berpotensi untuk menyara serovar *hardjo*. *Leptospira interrogans* serovar *hardjo* boleh terus hidup di air dan tanah di keadaan persekitaran Malaysia dan organisma ini dipercayai boleh disebarkan kepada haiwan yang mudah terkena jangkitan.

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ABTS	2, 2-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) Diammonium Salt
bp	base pair
BRENDA	Bacterial restriction endonuclease DNA analysis
BSA	Bovine serum albumin
cm	centimeter
DAB	diaminobenzidene
^{o}C	degree celcius
DNA	Deoxyribonucleic acid
EDTA	ethylene diamine tetra acetic
ELISA	Enzyme-linked immunosorbent assay
EMJH	Ellinghausen, McCullough, Johnson and Harris
5-FU	5-fluorouracil
G	gauge
H_2O2	Hydrogen peroxide
HCl	Hydrochloric acid
H & E	haematoxylin and eosin
HIS	Hyper immune serum
IgG	Immunoglobulin G
IgM	Immunoglobulin M

JS	Johnson and Seiter
Kbp	Kilobase pair
kDa	Kilodalton
M	Molar
MAT	Microscopic agglutination test
MARDI	Malaysian Agricultural Research and Development Institute
mM	Millimolar
ml	Milliliter
mmol/l	milimol per liter
mg/l	milligram per liter
mol/l	milimol per liter
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate –buffer saline
PCR	Polymerase chain reaction
pH	puissance hydrogen (Hydrogen-ion concentration)
p.i	post inoculation
RBC	Red blood cell
PCV	Pack cell volume
RDP	Rancangan Daging Penusu
rpm	round per minute
SDS	Sodium dodecyl sulfate

TBS	Tris buffer saline
Tris-HCL	Tris (hydroxymethyl) aminomethane hydrochloride
TEMED	N,N,N′,N′-Tetra-methylethylenediamine
UPM	Universiti Putra Malaysia
v/v	volume per volume
w/v	weight per volume
WBC	White blood cell
μ	micron
μg	microgram
μl	microlitre