UNIVERSITI PUTRA MALAYSIA

THE EPIDEMIOLOGY AND BACTERIOLOGY OF LEPTOSPIRAL INFECTION IN TWO SELECTED CATTLE FARMS IN PENINSULAR MALAYSIA

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By

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LIST OF ABBREVIATIONS

ABTS	2, 2'-AZINO-bis (3-ETHYLBENZTHIAZOLINE-6-SULFONIC ACID) Diammonium Salt
B. Arang	Batu Arang
BRENDA	Bacterial Restriction Endonuclease DNA Analysis
CAAT	Cross-agglutination Absorption Test
cDNA	Complementary DNA
CFT	Complement Fixation Test
DNA	Deoxyribonucleic Acid
D.W.	Distilled water
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
ESS	Erythrocyte Sensitizing Substance
FAT	Fluorescent Antibody Technique
Hg	Mercury
H_2O_2	Hydrogen peroxide
I.C.S.B.	International Committee on Systematic Bacteriology
ID	Identification
IgG	Immunoglobulin G
IgM	Immunoglobulin M
JS	Johnson and Seiter
kD	Kilodalton
LPS	Lipopolysaccharide
M	Molar
mA	Milliampere

M.A.R.D.I.	Malaysian Agricultural Research and Development Institute
MAT	Microscopic Agglutination Test
mM	Millimolar
OD	Optical density
O/N	Overnight
pH	puissance hydrogene (Hydrogen-ion Concentration)
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate-buffered Saline
POD	Peroxidase
RIA	Radioimmunoassay
rpm	round per minute
SDS	Sodium Dodecyl Sulfate
Sg. Siput	Sungai Siput
TM	Type-specific Main
TRIS-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
UPM	Universiti Pertanian Malaysia
V	Volt
VRI	Veterinary Research Institute
v/v	volume per volume
w/v	weight per volume
μ	Micron
μg	Microgram
μl	Microlitre

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Master of Science.

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A serological survey of cattle on two selected farms in Malaysia revealed that 87.7 percent (150/171) of the animals examined had leptospiral antibodies. Sixty percent of the positive sera had titres to two or more serovars. Overall, serovar australis was found to be the most frequent serovar affecting the animals in these two farms. Amongst the three age groups of cattle, the yearling group had the highest prevalence (62/64) of infection.

A bacteriological survey of cattle on the two farms revealed 11.5 percent (13/113) of the animals were leptospiruric. Almost all of the isolates (12/13) were isolated from the yearling group. The isolates belonged to either the Sejroe or Pomona serogroup. Representative isolates have been sent to

the Leptospirosis Reference Laboratory in Brisbane, Australia for definitive identification.

The enzyme-linked immunosorbent assay (ELISA) using genus-specific (sonicated) antigen was employed for the detection of leptospiral infection. It was found to be more sensitive than the microscopic agglutination test (MAT). However, it is only suitable for screening and epidemiological purposes.

The Coomassie blue-stained protein profiles of selected serovars examined by polyacrylamide gel electrophoresis (PAGE) were generally identical except for a few discernible differences but the silver-stained lipopolysaccharide (LPS) profiles were more distinguishable.

Almost all of the proteins were blotted during electrophoretic transfer from the polyacrylamide gels to nitrocellulose membranes and a majority of the protein bands that were detected on the membranes were shown to be immunogenic. A few of the proteins were serovar-specific but others appeared to be common amongst the serovars tested.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi keperluan Ijazah Master Sains.

EPIDEMIOLOGI DAN BAKTERIOLOGI JANGKITAN LEPTOSPIRA DI DUA LADANG TERNAKAN LEMBU TERPILIH DI SEMENANJUNG MALAYSIA

Oleh

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Satu banci ke atas ternakan lembu di dua buah ladang di Malaysia menunjukkan 87.7 peratus (150/171) ternakan yang di periksa mempunyai antibodi kepada leptospira. Enam puluh peratus serum mempunyai titer terhadap dua atau lebih serovar. Pada keseluruhannya, serovar australis adalah didapati yang sering sekali menjangkiti ternakan di kedua-dua ladang tersebut. Antara tiga kumpulan ternakan lembu berasas umur, kumpulan umur satu tahunan mempunyai prevalens jangkitan yang tertinggi (62/64).

Banci bakteriologi ke atas ternakan lembu di kedua-dua ladang mendapati 11.5 peratus (13/113) ternakan berkenaan berleptospirurik. Hampir semua isolat (12/13) yang telah diasingkan berasal dari kumpulan umur satu tahun. Isolat-isolat yang di asingkan didapati berasal dari serogroup Sejroe atau

Pomona dan wakil-wakil isolat telah dihantar ke Makmal Rujukan Leptospira di Brisbane, Australia untuk pengenalpastian muktamad.

Assai imunoserap terikat enzim menggunakan antigen khusus genus bersonikat telah digunakan untuk mengesan jangkitan leptospira. Assai ini didapati lebih peka daripada ujian pengaglutinatan mikroskop. Walaubagaimanapun, ia hanya sesuai untuk tujuan menapis jangkitan dan kajian epidemiologi.

Dalam elektroforesis poliakrilamid gel, profil protein serovar yang telah dipewarnakan dengan koomasie biru didapati serupa kecuali dua-tiga perbezaan. Sebaliknya, pewarnaan perak ke atas lipopolisakarid menunjukkan profil yang lebih banyak perbezaan.

Hampir semua protein telah dapat dipindahkan (blot) daripada gel poliakrilamid ke membran nitroselulosa. Setelah imunopewarnaan kebanyakan band protein didapati berimunogen. Hanya terdapat sebilangan protein yang khusus kepada serovar sementara banyak yang lain nampaknya, terdapat dalam semua serovar yang diuji.

CHAPTER I

INTRODUCTION

Importance of Leptospirosis in Malaysia

Leptospirosis is an important zoonosis in Malaysia. High incidence of the infection has been reported in rural workers and soldiers on jungle operations. Most human cases of leptospirosis are contracted directly or indirectly from animals. In domestic animals, leptospirosis is often inapparent; fever, inappetence and depression are the usual signs but still it is an important economic disease since animal health and productivity are affected. Abortions, stillbirths, weak progeny, mastitis and infertility have been attributed to leptospiral infections (Ellis *et al*., 1985).

Objectives of This Present Study

Extensive study by Bahaman *et al*. (1987) has established the distribution and prevalence of leptospirosis in domestic animals in Malaysia. Based on that study, cattle were shown to have the highest prevalence of leptospiral infection particularly in two farms; the Batu Arang Farm and the Sungai Siput Farm. Thus, these two cattle farms were selected to study the epidemiology of leptospiral infection. Moreover, these two herds were infected with multiple serovars. With regards to bacteriology, serovar *canicola* infection was found to be the

highest in Batu Arang Farm. It is not established whether canicola infection in cattle in that farm was endemic. Thus, this present study is a follow-up to determine the status of the infection in the two farms.

Both serological and bacteriological aspects of leptospirosis will be studied by conventional methods. Although the microscopic agglutination test (MAT) has been widely used, it is unable to detect certain antibodies in animal sera (Adler et al., 1981; Ellis et al., 1982) and the results are difficult to interpret with a possibility of low titres being non-specific or cross-reacting. MAT is also hazardous, time consuming and requires maintaining a battery of leptospiral antigens to perform. On the other hand, the enzyme-linked immunosorbent assay (ELISA) is rapid and simple to perform. The genus-specific ELISA will be useful for screening large number of sera.

Only a paucity of reports on leptospiral proteins and DNA were available (Marshall et al., 1981; Robinson et al., 1982; Nunes-Edward et al., 1985; Le Febvre et al., 1987). The introduction of special techniques: Polyacrylamide gel electrophoresis (PAGE) and Immunoblotting, will give more information on the protein profiles of different strains as well as indications of which proteins are immunogenic.

Three main objectives that have been identified are

1. to determine the prevalence of leptospiral infection in cattle on selected farms in Malaysia,

2. to evaluate the ELISA technique as a screening test, and
3. to study the protein profiles and immunogenicity of the leptospiral components by polyacrylamide gel electrophoresis and immunoblotting.

Fundamental work will therefore have to be carried out on the occurrence of leptospiral infection in cattle in the two selected farms. Sera obtained will be examined by both MAT and ELISA methods. Finally, leptospires isolated from urine samples will be studied on their protein composition and related immunogenicity.

Multiple leptospiral serovar infection of cattle in the two farms had been reported (Bahaman et al., 1988). Although, it is not established whether multiple serovar infection was due to actual endemic infection. Several leptospiral serovars were isolated from rats (Alexander et al., 1957; Gordon-Smith et al., 1961). Thus, the multiple serovar infection in cattle in the two farms was possibly the transitional infection from rats. This follow-up study will determine the circumstances that lead to the current infection and possibility of cross-reacting titres.

CHAPTER II

LITERATURE REVIEW

Leptospires

Morphology

Leptospires are bacteria with a characteristic helical (spiral) morphology. One or both ends of the cells are typically hooked. Leptospires are actively motile. Both their morphology and motility can be seen with the aid of a darkfield microscope and appear white in contrast to the background.

Classification

According to the latest Bergey's Manual(1984), the genus Leptospira has been divided into two species; Leptospira interrogans (the pathogenic leptospires) and L. biflexa (the saprophytic ones). Based on antigenic analysis, there are 25 serogroups currently recognised in L. interrogans (Dikken, 1986). All serovars which cross agglutinate to a high titre with one anothers' antisera are placed into a common serogroup. Almost 200 serovars of leptospires have been identified throughout the world (Neill et al., 1986). Out of this large number of serovars, 37 are at present known to occur in Malaysia (Table 1).

Table 1

Leptospiral Serovars Isolated from
Animals and Man in Malaysia

Serogroup	Serovar	Host	Reference
1. Australis	australis	Cattle	Bahaman & Ibrahim (1986)
		Man	Alexander et al.(1957)
		Rats	Gordon-Smith et al.(1961)
	fugis	Man	Alexander et al.(1957)
2. Autumnalis	autumnalis	Man	Alexander et al.(1957)
			Tan (1970)
	bangkinang	Man	Alexander et al.(1957)
	djasiman	Rats	Gordon-Smith et al.(1961)
	gurungi	Man	Alexander et al.(1957)
	mooris	Man	Alexander et al.(1957)
	sentot	Palm-civet	Gordon-Smith et al.(1961)
	unknown	Man	Fletcher (1928)
		Dogs	Fletcher (1928)
		Rats	Gordon-Smith et al.(1961)
3. Bataviae	bataviae	Man	Alexander et al.(1957)
		Rats	Gordon-Smith et al.(1961)
	paidjan	Rats	Alexander et al.(1957)
	unknown	Rats	Gordon-Smith et al.(1961)

Table 1 (continued)

Serogroup	Serovar	Host	Reference
4. Canicola	benjamin	Rats	Gordon-Smith et al.(1961)
	canicola	Cattle	Bahaman & Ibrahim (1986)
		Man	Alexander et al.(1957)
			Tan (1970)
		Rats	Gordon-Smith et al.(1961)
	jonsis	Man	Alexander et al.(1957)
	malaya	Man	Alexander et al.(1955)
	schuffneri	Man	Alexander et al.(1957)
		Rats	Alexander et al.(1955)
	sumner	Man	Alexander et al.(1957)
5. Celledoni	celledoni	Man	Alexander et al.(1957)
		Rats	Gordon-Smith et al.(1961)
	whitcombi	Rats	Alexander et al.(1957)
6. Grippoty-phosa	grippoty-phosa	Man	Alexander et al.(1957)
			Tan (1970)
		Rats	Alexander et al.(1955)
7. Hebdomadis	hebdomadis	Man	Tan (1970)
	worsfoldi	Man	Alexander et al.(1957)
	unknown	Palm-civet	Gordon-Smith et al.(1961)
		Rats	Gordon-Smith et al.(1961)

Table 1 (continued)

Serogroup	Serovar	Host	Reference
8. Icterohemo-rrhagiae	birkini	Man	Alexander et al.(1957)
	icterohemo-rrhagiae	Man	Tan (1970)
	mankarso	Man	Alexander et al.(1955)
	smithii	Man	Alexander et al.(1957)
	unknown	Dog	Gordon-Smith et al.(1961)
		Man	Fletcher (1928)
		Rats	Gordon-Smith et al.(1961)
9. Javanica	coxis	Man	Alexander et al.(1957)
	javanica	Cattle	Bahaman & Ibrahim (1986)
		Rats	Gordon-Smith et al.(1961)
	unknown	Rats	Gordon-Smith et al.(1961)
10.Pomona	pomona	Cat	Gordon-Smith et al.(1961)
		Cattle	Bahaman et al.(1988)
		Man	Alexander et al.(1957)
			Tan (1970)
		Palm-civet	Gordon-Smith et al.(1961)
11.Pyrogenes	abramis	Man	Alexander et al.(1957)
	biggis	Man	Alexander et al.(1957)
	hamptoni	Man	Alexander et al.(1957)

Table 1 (continued)

Serogroup	Serovar	Host	Reference
	pyrogenes	Man	Alexander et al.(1957)
			Tan (1970)
	unknown	Man	Alexander et al.(1957)
12.Sejroe	hardjo	Cattle	Bahaman et al.(1988)
	hemolytica	Man	Alexander et al.(1957)
	ricardi	Man	Alexander et al.(1957)
	unipertama	Cattle	Bahaman et al.(1990)
	wolffi	Man	Alexander et al.(1957)
13.Tarassovi	unknown	Man	Fletcher (1928)
		Rats	Gordon-Smith et al.(1961)

Transmission

Antibodies to leptospires have been detected in a great variety of wild and domestic animal species in various countries. Fortunately, only a few animal species are able to maintain the leptospires in their kidneys and act as chronic carriers, shedding the organisms in their urine for several months. The important reservoirs of leptospiral infection are rodents, cattle, pigs and dogs (Christmas et al., 1974; Everard et al., 1979). The increase in stocking rates associated with