UNIVERSITI PUTRA MALAYSIA

A SEROLOGICAL AND BACTERIOLOGICAL STUDY OF LEPTOSPIRAL INFECTION IN DOMESTIC ANIMALS IN PENINSULAR MALAYSIA

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FPV 1988 5

A SEROLOGICAL AND BACTERIOLOGICAL STUDY OF LEPTOSPIRAL INFECTION IN DOMESTIC ANIMALS IN PENINSULAR MALAYSIA

by

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A thesis submitted in partial fulfilment of the
requirements for the degree of Doctor of Philosophy
in the Faculty of Veterinary Medicine and Animal Science
Universiti Pertanian Malaysia

March 1988

ACKNOWLEDGMENTS

I am indebted to Professor Dr. Abdul Latif Ibrahim, Dean of the Faculty of Veterinary Medicine and Animal Science, UPM for granting me the opportunity to pursue this study. As my supervisor, he has given me invaluable guidance, encouragement and made constructive criticisms throughout the course of this study.

I am also very grateful to Universiti Pertanian Malaysia for the financial assistance that has helped make this study possible.

My special thanks to Dr. Roger B. Marshall, Department of Veterinary Pathology and Public Health, Massey University, New Zealand who during his visit to UPM, has given valuable comments and suggestions and with whom I have had stimulating discussions on the subject.

I would also like to thank various individuals and institutions who have helped me during the actual study and preparation of this thesis:

The Director-General of the Department of Veterinary Services, Minisitry of Agriculture, Malaysia for permission to procure blood and urine samples from various farms and abattoirs throughout the country and the various farm managers and workers who have assisted me with the work,

Dr. Dora S. K. Tan of the Institute of Medical Research, Kuala Lumpur for the supply of leptospiral antigens,

Professor Ryo Yanagawa, Department of Hygiene and Microbiology, Hokkaido University, Japan for giving me the opportunity to work in his laboratory and making available facilities for research. The work on ELISA was performed at his laboratory,

Dr. Etsuro Ono and others, also from the same Department for helping me in my work and making my stay in Sapporo a pleasant and memorable one,

Mr. N.D. Stallman and Mr R.D. Tinniswood of the Leptospirosis Reference Laboratory, Brisbane, Australia and Dr. A.F. Kaufmann of the Leptospirosis Reference Laboratory, Center for Disease Control, Atlanta, USA for definitive identification of my leptospiral isolates,

Mr. Hamzah Adam for his technical assistance, Mrs. Josephine Low for typing the tables and figures seen in this thesis and all friends whose names I am unable to mention personally, for their assistance and encouragement which made the going easier.

I owe a special debt of gratitude to my wife, Mazidah who has given me so much motivation, inspiration and loving support throughout the course of this study. To my wife and family whose love and understanding have greatly helped me in my studies, I dedicate this thesis.

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LIST OF ABBREVIATIONS

ABTS	2,2'- Azino-di(3-ethyl benzthiazoline sulfonic acid) diammonium salt
BJ	Bey and Johnson
BRENDA	Bacterial Restriction Enzyme Analysis
C	Celsius
CFT	Complement Fixation Test
DEAE	Diethylaminoethyl
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
FAT	Fluorescent Antibody Technique
H_2O_2	Hydrogen peroxide
g	Gram
Ig	Immunoglobulin
IgG	Immunoglobulin G
JS	Johnson and Seiter
KK	Kedah-Kelantan
LID	Local Indian Dairy
MARDI	Malaysian Agricultural Research and Development Institute
MAT	Microscopic Agglutination Test
mg	Milligram
ml	Millilitre
mM	Millimolar
pH	puissance hydrogene (Hydrogen-ion Concentration)

PBS	Phosphate-buffered Saline
POD	Peroxidase
PT	Pusat Ternakan
RIA	Radioimmunoassay
RPM	Revolution Per Minute
RTH	Rancangan Ternakan Haiwan
RT	Room Temperature
TM	Type-specific Main
ug	Microgram
ul	Microlitre
UPM	Universiti Pertanian Malaysia

An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

A SEROLOGICAL AND BACTERIOLOGICAL STUDY OF LEPTOSPIRAL INFECTION IN DOMESTIC ANIMALS IN PENINSULAR MALAYSIA

by

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March, 1988

Supervisor : Professor Dr. Abdul Latif Ibrahim

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A cross-sectional serological survey of domestic animals in West Malaysia revealed that 25.5 percent of the animals examined had agglutinating antibodies to one or more antigens belonging to Leptospira interrogans. Significant prevalence of infection was observed in cattle (40.5%), buffaloes (31%) and pigs (16%). The Sejroe and Pomona serogroups were the principal ones involved whilst infections to the other eight serogroups appeared insignificant and were indicative of sporadic infection. Majority of the large cattle and buffalo farms demonstrated high prevalences of leptospiral

infection. Amongst cattle, the droughtmasters had the highest prevalence whilst the Kedah-Kelantan (an indigenous breed) had the lowest prevalence of leptospiral infection. Leptospiral infection in goats and sheep was shown to be sporadic.

A bacteriological survey of cattle revealed 18.9 percent (42/222) had leptospiral infection. Isolates were obtained from all herds except one. Six leptospiral serovars, namely, canicola, australis, javanica, ballum, pomona and hardjo were isolated for the first time in cattle in Malaysia. An isolate obtained from a bovine kidney proved to be a new serovar and was given the name unipertama. The small number of buffalo urine samples examined were all negative on culture.

An enzyme-linked immunosorbent assay (ELISA) using purified type-specific main (TM) antigen was developed for the detection of leptospiral infection. It was found to be more sensitive than MAT and would be a suitable method for detecting antibodies to leptospiral infection in animals in diagnostic and epidemiological studies.

It appears that the epidemiology of leptospiral infection in domestic animals in Malaysia is similar to that reported overseas. Evidence obtained indicated that cattle and pigs in Malaysia are the maintenance host for serovars hardjo and pomona respectively. With the advent of sophisticated farming, domestic animals will play a

bigger role in the epidemiology of leptospiral infection and will be a primary source of infection to humans. Under the present Malaysian farming system, vaccination of cattle is not advisable but highly recommended for pigs. It is believed that leptospirosis in Malaysia is not amendable to eradication, primarily because of the prevalence and wide range of susceptible animal hosts, both wild and domestic.

Ringkasan tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi sebahagian keperluan Ijazah Doktor Falsafah.

SATU KAJIAN SERUM DAN BAKTERIA JANGKITAN LEPTOSPIRA DALAM TERNAKAN HAIWAN DI SEMENANJUNG MALAYSIA

Oleh

Abdul Rani Bahaman

March, 1988

Penyelia: Professor Dr. Abdul Latif Ibrahim

Fakulti: Kedoktoran Veterinar dan Sains Peternakan

Satu banci serum keatas ternakan haiwan di Malaysia Barat menunjukkan 25.5 peratus ternakan yang diperiksa mempunyai antibodi aglutinin kepada satu antigen Leptospira interrogans atau berbilang. Prevalens jangkitan penting yang terdapat dikalangan ternakan adalah lembu (40.5 %), kerbau (31 %) dan babi (16 %). Serokumpulan Sejroe dan Pomona adalah kumpulan leptospira yang terpenting. Jangkitan kepada lapan serokumpulan lain nampaknya tidak penting dan sebaliknya menunjukkan jangkitan sporadik. Kebanyakkan ladang-ladang lembu dan kerbau yang diperiksa menunjukkan prevalens jangkitan leptospira yang tinggi. Di kalangan lembu, jenis droughtmasters mempunyai prevalens tertinggi dan jenis Kedah-Kelantan (baka

tempatan) mempunyai prevalens terendah sekali. Jangkitan leptospira di kalangan kambing biasa dan kambing bebiri merupakan jangkitan sporadik sahaja.

Satu banci bakteria leptospira keatas lembu-lembu, menunjukkan 18.9 peratus (42/222) haiwan yang diperiksa mempunyai jangkitan leptospira. Enam serovar leptospira, iaitu canicola, austalis, javanica, ballum, pomona dan hardjo telah dapat dipencilkan dari ternakan lembu bagi pertama kali nya di Malaysia. Satu isolat dari buah pinggang lembu merupakan serovar terbaru dan diberikan nama unipertama. Bilangan kecil air kencing kerbau yang diperiksa didapatai bebas dari leptospira.

Satu cara asai imunoresapan terikat enzim (AITE) yang menggunakan antigen utama khusus tip dan tulin telah direka untuk mengesan jangkitan leptospira. Asai ini didapati lebih sensitif daripada UAM dan adalah satu kaedah yang sesuai untuk mengesan antibodi dalam haiwan bagi diagnosis dan epidemiologi.

Adalah didapati bahawa epidemiologi jangkitan leptospira dalam ternakan haiwan di Malaysia ini serupa dengan yang berlaku dalam ternakan haiwan di luar negeri. Tanda-tanda menunjukkan ternakan lembu dan babi di Malaysia menanggung jangkitan hardjo dan pomona. Dengan adanya sistem penternakan yang bertaraf tinggi kelak, ternakan haiwan akan memain peranan penting dalam epidemiologi jangkitan leptospira dan berlaggak sebagai sumber-sumber jangkitan

primer kepada manusia. Dibawah sistem peternakan yang terdapat di Malaysia sekarang, pengvaksinan kepada lembu-lembu tidaklah digalakkan tetapi amat bersesuaian bagi ternakan babi. Adalah dipercayai, leptospirosis di Malaysia tidak akan dapat dihapuskan olih kerana terdapatnya prevalens jangkitan yang tinggi dan banyak pula hos haiwan ternakan dan liar yang mudah kena jangkitan.

CHAPTER 1

INTRODUCTION

Organisms which belong to the genus Leptospira are long, thin, helical (spiral) bacteria which can best be seen by darkfield microscopy. These flexible cells are about 0.1 to 0.3 um in diameter and from 6 to over 12 um in length. One or both ends of the cells are typically hooked. Their movement is very characteristic and appears as an alternating rotation around the long axis and translation in the direction of the unhooked cell end (Bergey's Manual, 1984). Thus, the motility differs from that of other bacteria and provides one of the characteristic diagnostic features of leptospires. All leptospires are structurally indistinguishable and can be differentiated into strains or serovars by immunological techniques.

Leptospirosis is recognised as an important disease of animals and man throughout the world and it has been classified as an important zoonotic disease. All human cases of leptospirosis are contracted directly or indirectly from animals. Leptospirosis in animals causes important, but often vaguely defined, economic losses to the livestock industry. Although this disease is usually mild and often subclinical, it can produce abortions, stillbirths, retained placenta, weak progeny, mastitis, and, with certain leptospiral serovars, death (Songer et al., 1983). An increasing awareness of

the economic importance of leptospirosis, particularly in domestic animals and their transmissibility to man has resulted in diagnostic facilities being made available in many countries. Outbreaks of haemoglobinuria or abortions in pigs in Malaysia have drawn attention to the widespread occurrence of leptospiral infection in animals in this country (Brandenburg and Too, 1981), (Joseph, 1979). Investigations of human leptospirosis are being carried out at the Institute of Medical Research, Kuala Lumpur whilst the Veterinary Research Institute, Ipoh and Universiti Pertanian Malaysia, Serdang undertake research on animal leptospirosis.

Classification and Nomenclature

For many years the genus *Leptospira* has been divided into two main groups, the pathogens and the saprophytes. Later, various test reactions have been reported to distinguish these two groups and the Taxonomic Subcommittee on Leptospira (1963) recommended that they be regarded as two species, *Leptospira interrogans* (the pathogenic ones) and *Leptospira biflexa* (the saprophytic leptospires). The reliability and reproducibility of some of the tests were later doubted and none was found sufficiently reliable for classification (Kmety *et al.*, 1966). The proposal by Borg-Petersen (1966) that there is only one species in the genus *Leptospira*, was adopted by the W.H.O. Expert Group (1967) and by the Subcommittee on the taxonomy of *Leptospira*. Thus, in the eighth edition of Bergey's Manual (1974), only one species of *Leptospira* was listed. However,

in the latest Bergey's Manual (1984), this has once again reverted to two species, Leptospira interrogans and Leptospira biflexa. A third species, Leptospira illini, has an uncertain taxonomic status and is listed as a species incertae sedis, however it has been proposed that, because of morphological differences which distinguish this organism from other leptospires, it should be placed in a new genus (Leptonema) (Hovind-Hougen, 1979).

Numerous serovars based on antigenic composition have been identified within the two species of Leptospira. This antigenic analysis is accomplished by microscopic agglutination and the cross-agglutinin absorption test which requires a considerable amount of experience to interpret and its use is restricted largely to leptospirosis reference laboratories.

All the serovars which cross agglutinate to a high titre with one anothers' antisera are placed into a common serogroup. There are 25 serogroups currently recognised (Dikken, 1986). These serogroups have a practical value as they help to reduce the number of antigens used in screening unknown sera. The serovars of Leptospira are not regarded as species. Nevertheless, they are still commonly designated in publications by the use of the binominal convention which has been reserved, under the rules of the International Code of Nomenclature of Bacteria and Viruses (1966) for designating species. Thus, for example, Leptospira interrogans serovar canicola is designated serovar canicola or simply canicola. The current method for identifying leptospiral serovars is by cross-agglutination absorption but this method does not differentiate

between strains within a serovar (Hathaway et al., 1985). The introduction of restricted endonuclease analysis as a technique for identifying leptospires (Marshall et al., 1981) has provided a reproducible method of demonstrating differrences in DNA composition between strains. This method consists of extracting protein-free DNA from a homogenous population of leptospires, digestion of the DNA with a restriction endonuclease, and electrophoresis of the digested DNA in an agarose gel. Because restriction endonucleases recognise and cleave double-stranded DNA at specific 4 or 6 base-pair sequences, a set of fragment is generated. The migration of these fragments in agarose gel is related to molecular weight; a pattern of bands is produced that can be seen in the gel by ultraviolet light if stained with ethidium bromide.

Based on immunological techniques, almost 200 serovars of leptospires from 19 serogroups have been identified throughout the world (Faine, 1982; Hathaway et al., 1983; Johnson, 1976), Out of this large number of serovars, 37 are at present known to occur in Malaysia (Table 1.1). Considering the warm and wet climate which is conducive to the growth and spread of leptospires, more leptospiral serovars are expected to be discovered in Malaysia. Bahaman and Ibrahim (1986) have isolated three leptospiral serovars, canicola, australis and javanica from a group of heifers. All three serovars were found to infect the one group of animals at the same time.