



UNIVERSITI PUTRA MALAYSIA

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

KUMLUNG CHUMPONBUNCHORN

FPV 1991 2

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

By

KUMLUNG CHUMPONBUNCHORN

**Thesis Submitted in Fulfilment of the Requirements for
the Degree of Master of Science in the Faculty of
Veterinary Medicine and Animal Science
Universiti Pertanian Malaysia**

May 1991



ACKNOWLEDGEMENTS

I would like to express my utmost appreciation and gratitude to my supervisor, Dr. Abdul Rani Bahaman, for his invaluable guidance, discussion and suggestions throughout the course of this study.

I am also grateful to the Faculty of Veterinary Medicine and Animal Science, Universiti Pertanian Malaysia for providing the research facilities.

A note of thanks to the Southeast Asian Ministers of Education Organization Regional Center For Graduate Study and Research in Agriculture (SEARCA) who has supported my study and the Department of Livestock Development, Thailand for granting me leave to be in Malaysia.

Lastly but not least, to my beloved wife who has always been understanding and given me the courage to complete this study.



TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vii
LIST OF FIGURES	ix
LIST OF PLATES	x
LIST OF ABBREVIATIONS	xi
ABSTRACT	xiii
ABSTRAK	xv
 CHAPTER	
I INTRODUCTION	1
Importance of Leptospirosis in Malaysia	1
Objectives of This Present Study	1
II LITERATURE REVIEW	4
Leptospire	4
Morphology	4
Classification	4
Transmission	8
Pathogenesis	10
Clinical Signs and Symptoms	11
Laboratory Diagnosis	12
Control and Prevention	14
Leptospirosis in Malaysia	15
Leptospirosis in Livestock in Malaysia	16



	Leptospirosis in Pet Animals in Malaysia ..	21
	Leptospirosis in Wildlife in Malaysia	21
	Leptospirosis in Man in Malaysia	22
	Special Techniques on Leptospire	23
	Enzyme-linked Immunosorbent Assay (ELISA) ..	23
	Polyacrylamide Gel Electrophoresis (PAGE) ..	24
	Electrophoretic Transfer of Proteins	25
III	THE SEROLOGICAL PREVALENCE OF LEPTOSPIRAL INFECTION IN SELECTED CATTLE FARMS IN PENINSULAR MALAYSIA	29
	Introduction	29
	Objectives of the Study	30
	Materials and Methods	31
	Results	34
	Discussion	37
IV	THE BACTERIOLOGICAL PREVALENCE OF LEPTOSPIRAL INFECTION IN SELECTED CATTLE FARMS IN PENINSULAR MALAYSIA	39
	Introduction	39
	Objectives of the Study	41
	Materials and Methods	42
	Results	50
	Discussion	54
V	THE DEVELOPMENT AND EVALUATION OF A GENUS- SPECIFIC ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF ANTIBODY AGAINST <u>LEPTOSPIRA INTERROGANS</u> IN CATTLE	58
	Introduction	58
	Materials and Methods	60



	Results	63
	Trial A: ELISA Using Sonicated and Boiled Antigen	63
	Trial B: The Specificity and Sensitivity of ELISA on MAT-positive and MAT-negative Bovine Sera	67
	Trial C: ELISA Using Five Different Sonicated Antigens	69
	ELISA on Field Sera	76
	Discussion	77
VI	ELECTROPHORETIC CHARACTERIZATION OF LIPO- POLYSACCHARIDE AND PROTEIN EXTRACTS OF SELECTED LEPTOSPIRAL SEROVARS	81
	Introduction	81
	Materials and Methods	82
	Results	86
	Protein Profiles of Eleven Leptospiral Serovars Representing Seven Leptospiral Serogroups	86
	Comparison of the Protein Profiles of Leptospiral Serovars from a Serogroup	89
	Lipopolysaccharide (LPS) Profiles of Three Leptospiral Serovars	92
	Discussion	93
VII	ELECTROPHORETIC TRANSFER OF LEPTOSPIRAL PROTEINS FROM POLYACRYLAMIDE GELS TO NITROCELLULOSE SHEETS	95
	Introduction	95
	Materials and Methods	96
	Results	101
	Discussion	105



VIII	GENERAL DISCUSSION	108
	The Prevalence of Leptospiral Infection in Selected Cattle Farms in Peninsular Malaysia ..	108
	Evaluation of the ELISA as a Screening Test for Detection of Leptospiral Infection	109
	PAGE and Immunoblotting for Analysis of Proteins, LPS and Immunogens of Leptospire ..	111
IX	CONCLUSION	113
	BIBLIOGRAPHY	116
	APPENDICES	127
	APPENDIX A: MEDIUM	127
	APPENDIX B: SUBSTRATE	132
	APPENDIX C: ADDITIONAL TABLES	134
	APPENDIX D: STAINING	143
	VITA	150



LIST OF TABLES

Table		Page
1	Leptospiral Serovars Isolated from Animals and Man in Malaysia	5
2	Serological Prevalence of Leptospiral Infection in Domestic Animals in Peninsular Malaysia	20
3	Serological Prevalence of Leptospiral Infection in Selected Cattle Farms in Malaysia	35
4	The Distribution of MAT-titres to 8 Leptospiral Antigens in Cattle from the Two Selected Farms in Malaysia	35
5	The Distribution of Leptospiral Infections According to Age-groups of Cattle	36
6	Comparison of Serological Prevalence Tested by MAT and ELISA	37
7	Bacteriological Prevalence of Leptospiral Infection in Cattle in the Selected Farms	50
8	Growth of Leptospire in Relation to Urine Samples	51
9	Bacteriological Prevalence of Leptospiral Infection in Individual Age-groups	52
10	Comparison of Bacteriological and Serological Prevalence of Infection as well as Serogroups Involved between Past and Present Surveys	53
11	Optical Density of Negative Bovine Sera	67
12	Comparison between the ELISA and MAT Titres of Positive Bovine Sera	75
13	Determination of Sensitivity and Specificity of ELISA for the Detection of Leptospiral Antibodies in Bovine Sera	76



Table		Page
14	Optical Density and Titres of Rabbit Anti-leptospires Sera in ELISA Using Sonicated <u>australis</u> Antigen	135
15	Optical Density and Titres of Rabbit Anti-leptospires Sera in ELISA Using Boiled <u>australis</u> Antigen	136
16	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>australis</u> Antigen	137
17	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>australis</u> Antigen	138
18	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>canicola</u> Antigen	139
19	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>hardjo</u> Antigen	140
20	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>javanica</u> Antigen	141
21	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>pomona</u> Antigen	142



LIST OF FIGURES

Figure		Page
1	The Procedure Involved in Culturing Urine Samples	43
2	Optical Density and Titres of Rabbit Anti-leptospire Sera in ELISA Using Sonicated <u>australis</u> Antigen	65
3	Optical Density and Titres of Rabbit Anti-leptospire Sera in ELISA Using Boiled <u>australis</u> Antigen	66
4	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>australis</u> Antigen	68
5	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>australis</u> Antigen	70
6	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>canicola</u> Antigen	71
7	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>hardjo</u> Antigen	72
8	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>javanica</u> Antigen	73
9	Optical Density and Titres of MAT-positive Bovine Sera in ELISA Using Sonicated <u>pomona</u> Antigen	74
10	Assembly for Electrophoretic Blotting Procedure	97



LIST OF PLATES

Plate		Page
1	Comparison of Coomassie Blue-stained Protein Profiles of Various Leptospiral Serovars by SDS-PAGE	88
2	Comparison of Coomassie Blue-stained Protein Profiles of Leptospiral Serovars in Individual Serogroups by SDS-PAGE	90
3	Comparison of Coomassie Blue-stained Protein Profiles of Leptospiral Serovars in Individual Serogroups by SDS-PAGE	91
4	Comparison of Silver-stained LPS Profiles of Three Serovars Representing Different Serogroups by SDS-PAGE	92
5	Coomassie Blue-stained Polyacrylamide Gel	101
6	Electrophoretic Blots of Leptospiral Protein (Isolate 0236) Stained with Dye	102
7	Electrophoretic Blots of Isolate 0236 Antigens Detected by Various Antisera	103
8	Electrophoretic Blots of Isolate 0236 Antigens Titrated with Homologous Antiserum at various Dilutions	104



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 ₂ O 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	<u>puissance hydrogene</u> (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
μ g	Microgram
μ l	Microlitre



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

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

By

KUMLUNG CHUMPONBUNCHORN

May 1991

Supervisor: Dr. Abdul Rani Bahaman

Faculty : Veterinary Medicine and Animal Science

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

KUMLUNG CHUMPONBUNCHORN

Mei 1991

Penyelia: Dr. Abdul Rani Bahaman

Fakulti: Kedokteran Veterinar dan Sains Peternakan

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, leptospire isolates 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

Leptospire

Morphology

Leptospire are bacteria with a characteristic helical (spiral) morphology. One or both ends of the cells are typically hooked. Leptospire 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 leptospire) 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 another's antisera are placed into a common serogroup. Almost 200 serovars of leptospire 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	<u>australis</u>	Cattle	Bahaman & Ibrahim (1986)	
		Man	Alexander <u>et al.</u> (1957)	
		Rats	Gordon-Smith <u>et al.</u> (1961)	
	<u>fugis</u>	Man	Alexander <u>et al.</u> (1957)	
2. Autumnalis	<u>autumnalis</u>	Man	Alexander <u>et al.</u> (1957) Tan (1970)	
	<u>bangkinang</u>	Man	Alexander <u>et al.</u> (1957)	
	<u>djasiman</u>	Rats	Gordon-Smith <u>et al.</u> (1961)	
	<u>gurungi</u>	Man	Alexander <u>et al.</u> (1957)	
	<u>mooris</u>	Man	Alexander <u>et al.</u> (1957)	
	<u>sentot</u>	Palm-civet	Gordon-Smith <u>et al.</u> (1961)	
	unknown		Man	Fletcher (1928)
			Dogs	Fletcher (1928)
Rats			Gordon-Smith <u>et al.</u> (1961)	
3. Bataviae	<u>bataviae</u>	Man	Alexander <u>et al.</u> (1957)	
		Rats	Gordon-Smith <u>et al.</u> (1961)	
	<u>paidjan</u>	Rats	Alexander <u>et al.</u> (1957)	
unknown	Rats	Gordon-Smith <u>et al.</u> (1961)		

Table 1 (continued)

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

Table 1 (continued)

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

Table 1 (continued)

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

=====
Transmission

Antibodies to leptospire 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 leptospire 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