

## LEPTOSPIROSIS IN ANIMALS IN WEST MALAYSIA

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### INTRODUCTION

Leptospirosis in man and animals is caused by the pathogenic species *Leptospira interrogans* and is characterised by a variety of syndromes. The pathogenic *L. interrogans* encompasses over a hundred serotypes which are grouped under 18 serogroups on the basis of their antigenic characteristics.

Pathogenic leptospires occur naturally in a wide variety of wild and domesticated animals throughout the world and is probably the most widely prevalent zoonoses(1). Natural reservoirs of infection are rodents and a large variety of other feral and domestic animals. Many serotypes occur predominantly in select mammalian hosts; however, the distribution of a specific serotype in a select host is not exclusive.

Generally the disease is more prevalent in areas where the climate is warm and humid, soils are alkaline and where there is heavy rainfall and consequent abundance of surface water and stagnant pools. The disease appears to occur more frequently in areas of extensive rice or sugar-cane cultivation(2).

Infections are incurred by contact with urine of carriers or indirectly by contact with streams, ponds, swamps or wet soils contaminated with urine of carriers. Pathogenic leptospires can survive for 3 months or longer on neutral or slightly alkaline waters but do not persist in brackish or acid waters.

In domestic animals the disease, in most cases, runs a sub-clinical course. It is, however, of economic importance as it causes losses due to deaths, especially in the young, abortions, stillbirths, weak newborns and neonatal mortality, loss in breeding efficiency and loss in milk production. The disease occasionally assumes a more severe clinical character and is manifested as outbreaks of leptospirosis or as an "abortion storm".

### EARLY REPORTS OF LEPTOSPIROSIS IN ANIMALS IN MALAYSIA

The Federation of Malaya Report of the Veteri-

nary Department for 1950 reported a case of *canicola* infection in an Army tracker dog from Johore(3). Subsequent to that leptospirosis in dogs was diagnosed on clinical and serological findings in Johore and Selangor. *Canicola*, *icterohaemorrhagiae* and pyrogenes were the serotypes involved. These cases in the early fifties were perhaps not the first reports of leptospirosis in animals in this country. An earlier report by Fletcher in 1928(4) indicated the presence of the disease in rats.

In 1955 Wisseman Jr. et al.(5) reported the results of a serological and cultural survey of man and animals in urban, rural and jungle areas of Southeast Asia. Out of 176 sera of domestic animals tested, 55 or 31.2% were positive out of which 13 were positive to more than one serotype. Table I gives the results of the survey.

It is interesting to note that the reactor rate in horses was the highest. The numbers of sheep, pigs and buffaloes were small and conclusions cannot be drawn from the figures as to prevalence rates. Dogs would appear to be the next highest in prevalence followed by cattle and goats.

The same workers tested 25 sera from rodents and found 4 of them positive. Three species of Malayan rodents, *Rattus mulleri*, *R. rajah* and *R. sabanus* were also culturally positive with the isolation of serotypes *hebdomadis*, *grippotyphosa* and *schuffneri*.

In 1961 Smith et al.(6) in their serological survey of domestic animals in Malaya obtained the following reactor rates:—

goats	— 28%
dogs	— 18%
buffaloes	— 15%
pigs	— 11%
cats	— 10%
oxen	— 4%
birds	— 0%

The dominant serogroups in domestic animals as detected by the serological test were Hebdomadis, Icterohaemorrhagiae, Javanica

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TABLE I  
SURVEY OF LEPTOSPIRAL ANTIBODIES IN MALAYAN  
DOMESTIC ANIMALS – (WISSEMAN *et al.*, 1955)

<i>Animal</i>	<i>No. Tested</i>	<i>No. Reactors</i>	<i>No. Multiple Reactors</i>	<i>Antigen giving positive reactions*</i>
Goats	13	2 (15.4)	0	2, 4
Sheep	5	1	0	1
Pigs	5	3	0	2, 9, 13
Horses	29	19 (65.5)	7	1, 4, 2, 8, 9, 3, 5, 6, 10, 11
Cattle	98	21 (21.4)	5	1, 12, 3, 2, 4, 8, 9
Buffalo	7	1	0	1
Dogs	19	8 (42.1)	1	4, 8, 1, 7, 10

\*Code numbers of leptospiral antigens are listed in order of frequency and refer to the following serotypes: 1 – *hebdomadis*; 2 – *autumnalis*; 3 – *pyrogenes salinem*; 4 – *canicola*; 5 – *icterohaemorrhagiae*; 6 – *bataviae*; 7 – *grippot yphosa*; 8 – *semeranga*; 9 – *pornona*; 10 – *djasiman*; 11 – *ballum*; 12 – *schuffneri*; 13 – *sentot*.

Note: Figures in parentheses indicate percentages.

and *Canicola*. All these were also commonly associated with rodents. Contrary to the previous survey by Wisseman *et al.* in 1955(5), the survey by Smith *et al.*(6) in 1961 had goats giving the highest prevalence with cattle the lowest among domestic mammals.

The survey of Smith *et al.*(6) also included a wide variety of rodents and wild carnivores from different habitats and the results indicated that forest ground rats had a prevalence of 14% as compared to forest tree rats 1%, house rats and mice 9% and rats of shrub and cultivation 12%. Home shrews had a prevalence rate of 5% as compared to 3% in tree shrews. Wild carnivores and reptiles gave prevalence rates of 16% and 14% respectively. The most frequent leptospires isolated from the above were serotypes *icterohaemorrhagiae* and *javanica*.

In 1969 the Veterinary Research Institute (VRI) submitted 233 cattle sera from government farms in Johore and Negeri Sembilan and 169 pig sera from government and commercial pig farms in Selangor to the United States Army Medical Research Unit (USAMRU) at the Institute for Medical Research (IMR), Kuala Lumpur. The results of the Haemolytic Lysis (HL) test conducted on these sera indicated that 19 or 8.1% of the cattle sera and 13 or 7.7% of the pig sera gave titres 1:40 and above. Six of the 19 cattle reactors gave titres 1:160

and over. These sera were all collected from apparently healthy stock and the purpose of the serological survey was to establish the status of leptospirosis in these 2 species of animals. A similar attempt was made to evaluate water buffaloes in the country. Ninety(90) buffalo sera from various parts of the country were collected by the VRI and submitted to Prof. Y.P. Liu of the National Taiwan University. Only 1 out of the 90 sera gave a titre of 1:100 to the Microscopic Agglutination Test (MAT) for serotype *icterohaemorrhagiae*. One would have expected a higher prevalence rate in water buffaloes because of their close association with padi fields, stagnant pools of water and mud. The surveys conducted indicated that domestic animals (with the exception of birds), wild carnivores and rodents in this country had previous exposure to various serotypes of *Leptospira*. Some of them were also found to be carriers.

In veterinary staff and other occupational hazard groups, surveys were conducted in 1955 by Wisseman *et al.*(5) and in 1970 by Dora Tan. The earlier study involved 27 adult Malay, Chinese and Indian abattoir workers, butchers, and tanners of which 7 were found to be reactors and out of which 3 reacted to more than one serotype. Dr. Dora Tan's work (unpublished, 1970) covered veterinary staff in 7

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TABLE II  
RESULTS OF LEPTOSPIROSIS SEROLOGICAL SURVEY OF  
VETERINARY STAFF IN 7 MALAYSIAN STATES – (DORA TAN, 1970)

States	Total No. examined	No. positive	% positive
Selangor	64	28	44.0
Penang	32	1	3.1
•Kedah	18	0	0.0
Negeri Sembilan	46	3	6.5
Johor	44	1	2.3
*Trengganu	24	1	4.1
Pahang	20	3	15.0
Total	248	37	14.9

"Number of sera examined too small perhaps for results to be representative.

solidation of the lung, pale yellow miliary foci in the cortices of the kidneys and minute shallow ulcers in the gastric mucosa. Microscopy showed leptospire in renal tubules by the Warthin-Starry staining method and the presence of sub-acute interstitial nephritis.

In 1976 the RVDL at Bukit Tengah, Penang, conducted a leptospirosis survey on pig kidneys obtained from slaughtered pigs at the Penang abattoir. Condemned pig kidneys were collected over 5 slaughter days in May and June. One centimetre sections were cut and fixed in 10% buffered formalin immediately. After 24 hours, the tissues were processed and 5  $\mu$  thick sections stained with H & E and Warthin-Starry Silver Stain. (The average slaughter figure per day was 250–300 pigs and practically all were porkers and originated from farms in Penang island and Province Wellesley). Of the 31 kidneys examined 4 were positive for leptospire by the Warthin-Starry staining method. All 4 originated from 10 condemned kidneys in one sampling in June. The laboratory also investigated suspected clinical cases of leptospirosis in 1977 and confirmed the disease in 2 instances by histopathological examination and the elucidation of leptospire in renal tubules.

In late 1977 the RVDL, Petaling Jaya, investigated a report of abortions late in pregnancy in gilts over a period of 1 month in a farm in Rasah, Seremban. Six urine samples from the sows were examined under dark ground microscopy and large numbers of leptospire were seen in 2 of the samples. Serological

investigation of 8 affected sows indicated a predominantly *bataviae* infection (1 had 1:100 titre; 2 had 1:400 titre and 1 had a 1:1600 titre to the MAT). Of the 3 dogs in the farm one was in poor body condition and appeared lethargic and jaundiced. This dog gave a 1:100 titre to *bataviae* and *grippotyphosa* and a 1:400 titre to *cynopteri* and *canicola*.

#### Dogs

Canine leptospirosis is caused principally by *canicola*, *icterohaemorrhagiae* and infrequently by *pomona* serotypes. The diseases caused by the first two are clinically indistinguishable though classically the *icterohaemorrhagiae* infection is associated with severe liver involvement with icterus as the prominent feature while *canicola* infection is manifested mainly by kidney damage with consequent signs of renal failure(9). The illness caused by *pomona* is typically quite mild. In all probability the majority of cases of canine leptospirosis are subclinical. Those animals that do suffer a more severe illness experience severe vomiting, diarrhoea, widespread haemorrhage which may lead to anaemia, coughing, ocular and nasal discharges, impaired liver function, renal failure and death. Dehydration may be extreme and probably contributes to death. Dogs acquire *icterohaemorrhagiae* infection from carrier rats, but transmission of the *canicola* serotype is direct from dog to dog *via* contaminated urine.

Over a 2-year period 1977–78, 9 dogs were submitted to the VRI for investigation of

leptospirosis. Five of them gave positive titres to the MAT of which 2 were multiple reactors. Two of the dogs that died were autopsied and the histopathological findings of haemorrhagic gastroenteritis and acute interstitial nephritis were suggestive of leptospirosis. Both cases were negative on culture probably because tissues were collected 4–6 hours after death of the animals.

#### Horses

In horses and mules, leptospirosis is associated with an inflammatory condition of the eyes called Periodic Ophthalmia. The disease is characterized by sudden onset of acute symptoms (photophobia and lacrimation of one or both eyes, moderate pyrexia, catarrhal conjunctivitis, keratitis, opacity of the cornea) which subside but later recur following quiescent periods of various length. The etiology of Periodic Ophthalmia has been in dispute for many years. Nutrition studies indicate that there is an inverse relationship between incidence and the level of riboflavin in the diet. It has been shown that agglutination-lysis titres to especially pomona serotype are much higher and antibodies occur more frequently in the sera of horses affected with Periodic Ophthalmia. Elevated agglutination-lysis titres have also been shown to follow initial attacks of the disease. Leptospiral infection is clearly related to Periodic Ophthalmia, but the exact pathogenesis is not understood.

In a few thoroughbred race horses, opacities of the cornea in cases where there was no indication of external injury were checked for leptospirosis. In one of the cases the horse gave a titre of 1:100 to the pomona serotype but there was no elevation of the titre in subsequent tests. Clinical leptospirosis has never been recorded in horses in this country.

In the 4-year period 1975–78 the VRI conducted the MAT on 628 sera, including 13 from the laboratory staff. These sera came partly from animals in which leptospirosis was the clinical diagnosis or one of the differential diagnoses. Table III summarises the results obtained.

It is interesting to note that more than 50% of the reactors among cattle and pigs were

multiple reactors. Table IV gives the frequency of the various serogroups encountered. It appears that cattle in this country have encountered all 13 serogroups tested. Hebdomadis, Hyos and Pomona are the 3 most common serogroups involved in cattle. Pigs have antibodies against 6 serogroups of which the Bataviae, Ballum and Icterohaemorrhagiae serogroups appear to be more common.

Babudieri(2) reported that the serotype *hardjo* (Hebdomadis group), until recently a rare serotype and of only local interest, has now been found to be widely distributed in many countries causing infection in cattle and sheep. It certainly seems so in Malaysian cattle as the majority of sera reacting with the Hebdomadis serogroup gave significant titres to serotype *hardjo*. Isolation of serotype *hardjo* is reported to be difficult because it does not grow easily in the usual culture media. In New Zealand, Prof. J.A.R. Miles(11) reported that in the past the main cause of human infection had been serotype pomona but recent isolates have yielded serotype *ballum* and *Leptospira* of the Hebdomadis serogroup. There, the disease occurs predominantly in dairy workers. Summers et al.(12) in an infertility survey of beef cattle obtained a 73.7% and 80.0% reactor rate in 2 herds respectively to serotype *hardjo*. Michna and Campbell(13) in a survey of aborting cattle reported a 74.4% reactor rate to serotype *sejroe* (Hebdomadis serogroup) and isolated the organism from kidney of the aborting cows. The Hebdomadis serogroup was also isolated from aborted foetuses by Ellis and Michna(14).

Domestic animals now constitute the major source of infection in man. It seems likely that serotype *hardjo*, mainly adapted to cattle, and serotype pomona and perhaps serotype *tarasovi* (*hyos*) in cattle and pigs, act as sources of human infection, and it has been suggested that the incidence of serotype *hardjo* infection in man has increased significantly, parallel to the incidence in cattle(9, 15). With the rising incidence of leptospirae of the serogroups Hebdomadis, Hyos and Pomona in cattle in this country, we can expect to see a parallel increase of these serogroups in man in this country especially in dairy and abattoir workers.

TABLE III  
RESULTS OF LEPTOSPIROSIS MICROSCOPIC AGGLUTINATION TEST IN DOMESTIC ANIMALS AND VETERINARY LABORATORY STAFF, 1975-78.

<i>Animal species</i>	<i>No. Tested</i>	<i>No. Reactors</i>	<i>% Reactors</i>	<i>Multiple Reactors</i>
Cattle	495	175	35.3	96
Pig	52	12	23.0	7
Goat	26	2	7.7	1
Sheep	20	0	0	0
Horse	13	2	15.3	0
Dog	9	5	55.5	2
Veterinary Lab. staff	13	0	0	0
Total	628	196		106

TABLE IV  
FREQUENCY OF LEPTOSPIRAL SEROGROUPS IN DOMESTIC ANIMALS AS DETECTED BY THE MAT, 1975-78.

<i>No.</i>	<i>Serogroup</i>	<i>Species</i>					<i>Total</i>
		<i>Cattle</i>	<i>Pig</i>	<i>Goat</i>	<i>Horse</i>	<i>Dog</i>	
1.	Hebdomadis	78	—	—	1	—	79
2.	Hyos (Tarassovi)	74	—	—	—	—	74
3.	Pomona	58	2	—	1	—	61
4.	Icterohaemorrhagiae	11	6	1	—	2	20
5.	Cynopteri	13	3	—	—	1	17
6.	Bataviae	6	8	—	—	3	17
7.	Djasiman	15	—	—	—	—	15
8.	Ballum	4	8	2	—	1	15
9.	Australis	11	—	—	—	—	11
10.	Grippotyphosa	9	—	—	—	1	10
11.	Celledoni	8	—	—	—	—	8
12.	Pyrogenes	3	2	—	—	1	6
13.	Canicola	3	—	—	—	2	5

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