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Serological prevalence of leptospiral infection in domestic animals in West Malaysia

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SUMMARY

A cross-sectional serological survey of domestic animals in West Malaysia revealed that 25·5% 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 serogroup was shown to be the principal one involved in cattle and buffaloes, and to a lesser extent the Tarassovi and Pomona serogroups. Evidence of infection in domestic animals by strains bearing the other seven antigens appeared insignificant and was indicative of sporadic infection. A majority of the large (semi-intensive) cattle and buffalo farms demonstrated a high prevalence of leptospiral infection. In both species of domestic animals mentioned above, the prevalence of infection was significantly higher ($P = 0{\cdot}01$) in the semi-intensive farms than in the smallholdings. Amongst cattle, the droughtmasters had the highest prevalence whilst the Kedah-Kelantan (an indigenous breed) had the lowest prevalence of leptospiral infection. In general, the temperate breeds of cattle had a significantly ($P = 0{\cdot}01$) higher prevalence of infection than local breeds. Leptospiral infection in goats and sheep was shown to be sporadic, and the Pomona serogroup was the principal leptospiral serogroup involved in these small ruminants. The prevalence of infection in pigs was observed to decline during the study period, and it is suspected that pigs in West Malaysia are the maintenance host for serovar *pomona* whilst cattle are the maintenance host for serovar *hardjo*. Overall, it appears that domestic animals in Malaysia will play a bigger role in the epidemiology of leptospiral infection with the advent of sophisticated farming.

INTRODUCTION

Serological surveys for the presence of *Leptospira interrogans* antibodies in animals have been reported from almost every part of the world during the past 60 years (Amatredjo & Campbell, 1975; Michna, 1970). Lorey (1932) appears to be the first to record the serological examination of serum samples for evidence of leptospirosis. In his examination of porcine serum samples, Lorey found that nine of the samples tested had antibodies specific to serovar *icterohaemorrhagiae*. However, the first report of leptospirosis in cattle was from the USSR by Mikhin & Azhinov (1935), who reported the presence of antibodies to serovar

grippotyphosa (then known as serovar *bovis*) in calves recovering from acute infectious haemiglobinuria. Serological evidence was subsequently found for *grippotyphosa* infections in cattle in Australia, France, Germany and Japan (Amatredjo & Campbell, 1975).

One of the earliest records of leptospirosis in buffaloes is that by Pande *et al.* (1960), who reported that a number of buffaloes in India had signs of convulsion, prostration and high mortality due to serovar *hebdomadis* infection. A few years later, Kujumgiev (1963) demonstrated the presence of agglutinating antibodies to leptospiral infection in 31% (27/87) of the buffaloes examined in Bulgaria, where a concurrent outbreak of leptospirosis was seen in a group of cattle. Ryu (1969) examined 1146 buffaloes in Thailand, Taiwan and Malaysia for evidence of leptospiral infection; a significant number of reactors was seen in Taiwan (40%) and Malaysia (49·5%).

Little is known of leptospirosis in small ruminants. Wirth (1937) reported that goats in Austria were infected with serovar *icterohaemorrhagiae*, but the most important discovery was that by Van der Hoeden (1953), who showed the presence of clinical leptospirosis in goats due to serovar *grippotyphosa*. Though the infection reported by Van der Hoeden was latent, there was evidence of inappetence, icterus, haemoglobinuria, decrease in milk production, emaciation and abortion. Interstitial nephritis was one of the common lesions observed on necropsy. In general, serological surveys of leptospiral infection in sheep and goats from various countries have disclosed a low prevalence of infection (Blackmore, Bahaman & Marshall, 1982; Chappell, Hanson & Garrigus, 1961; van Riel & van Riel (1956). Schollum & Blackmore (1981) from their study concluded that goats are not a natural maintenance host for leptospires. Naturally occurring cases of leptospirosis in sheep have been described in New Zealand (Bahaman *et al.* 1980) and in the United States (Langham, Morse & Morter, 1958). Salisbury (1954) and Webster & Reynolds (1955) have described severe outbreaks of leptospirosis in sheep in New Zealand due to serovar *pomona*. The main clinical features reported were icterus, haemoglobinuria and high mortality. Inapparent infection was also a feature of the infection. In Bulgaria and the United States acute haemolytic infection, death and abortions due to leptospirosis were reported in sheep (Beamer, Hardenbrook & Morrill, 1953; Davidson & Hirsh, 1980; Zaharija & Todorovic, 1964).

Serological evidence of leptospiral infection in pigs has been reported by a number of workers (Woods *et al.* 1962), and leptospiral infection is now recognized as a major disease of pigs. It has been reported to cause reproductive failure in pigs in many countries, and the serovars commonly incriminated were *pomona*, *tarassovi* and *canicola*. Infection with these serovars may cause abortion, stillbirths and neonatal death of piglets (Michna, 1970). Sporadic infection with variable clinical symptoms has been reported with several other serovars, particularly *grippotyphosa* and *copenhageni* (Hanson, Reynolds & Evans, 1971). Leptospiral infection in pigs in England appears to differ from those reported in other countries. Serological investigations indicated absence of endemic infection with *pomona* and *tarassovi*, the two most common serovars prevailing in pig populations in many parts of the world (Hathaway & Little, 1981).

The first report of leptospirosis in domestic animals in Malaysia was a case in a dog (Fletcher, 1928), and the earliest attempt to examine the prevalence of

leptospiral infection in domestic animals in Malaysia was that conducted by Wisseman *et al.* (1955). The number of animals examined was quite small and the results obtained were apparently not truly representative of the disease occurrence. Limited studies on the prevalence of leptospirosis in cattle in Malaysia have shown that the majority of the animals examined had evidence of infection (Arunasalam, 1975; Joseph, 1979; Leong & Maamor, 1975). On the other hand, investigations on limited samples of pigs have indicated low (10·6%) prevalence of leptospiral infection (Gordon-Smith *et al.* 1961). Little or no information is available on leptospiral infection in the other species of domestic animals in West Malaysia. This present study is an attempt to determine the prevalence of leptospiral infection in the various species of domestic animals (except dogs and cats) in West Malaysia and to assess the significance of leptospiral infection in relation to the animal industry and public health in Malaysia. This cross-sectional serological study will help to determine the important leptospiral serovars involved, and indirectly help to extrapolate the epidemiological patterns of infection in the various species of domestic animal in West Malaysia. Overall, this is an attempt to assess the role of domestic animals in the epidemiology of leptospiral infection in West Malaysia.

MATERIALS AND METHODS

Various species of domestic animals in Malaysia were examined for evidence of agglutinating antibodies to the leptospiral antigens tested. Dogs and cats were excluded from this study; they were considered as pets or small animals and this study is confined to animals of economic importance, namely cattle, water buffaloes, goats, pigs and the few head of sheep found in West Malaysia.

Study herds

Blood samples were obtained from animals on government farms, smallholdings and from animals sent to the abattoirs for slaughter. There are only a few large government cattle, buffalo or goat farms found in the country. The smallholdings usually have only a few head of animals, which help to supplement the income of the farmers. Most of the serum samples obtained from cattle in this study were from yearlings, that is, animals of about 8 months to 2 years of age from the government farms. On the other hand, serum samples from smallholdings were obtained from animals of various ages (usually more than 2 years old). Animals sent to the abattoir were mainly males, as no healthy and productive females were allowed to be slaughtered under the government's strategy to preserve and increase livestock population. All animals sampled were apparently healthy at the time of collection of blood samples.

The serum samples from pigs were obtained from animals sent to the abattoir, and they were mainly porkers, animals of about 4 months of age. The buffaloes, sheep and goats sampled were mature animals, usually more than 2 years of age.

Sampling technique

Altogether 3377 blood samples were obtained during the study period, December 1981 to February 1986. Blood samples were obtained by venepuncture of the jugular or coccygeal vein using veneject (Terumo, Japan). In abattoirs, blood samples were obtained directly from the jugular vein into clean universal bottles immediately after the animal was slaughtered.

The first 50 or more animals that came into the stocking yard or chute were bled and the blood samples obtained. In abattoirs the number of animals, except for pigs, was small, so blood samples were obtained from every animal that was slaughtered. The blood samples were left to clot overnight in ice flasks or in the cold room if taken immediately to the laboratory. The serum was then pipetted out with a clean Pasteur pipette into a test tube. It was then centrifuged and the clarified serum sample obtained. Each serum sample was kept in a bijou bottle or in a 2 ml vial (Flowlab, Malaysia) and held at −20 °C until tested by the microscopic agglutination test (MAT) (Cole, Sulzer & Pursell, 1973).

Antigens

The antigens used in the MAT were made up of 4- to 8-day-old live culture of serovars *hardjo* (*Hardjo-prajitno*), *canicola* (*Hond Utrecht IV*), *icterohaemorrhagiae* (*RGA*), *celledoni* (*Celledoni*), *pyrogenes* (*Salinem*), *ballum* (*Mus 127*), *australis* (*Ballico*), *grippotyphosa* (*Moskva V*), *tarassovi* (*Perepelicin*) and *pomona* (*Pomona*) with estimated density of 10^8 organisms per millilitre. These leptospiral serovars were considered as the antigens for the MAT based on available scientific papers and reports from diagnostic laboratories in Malaysia. It was also based on the frequency of isolation and the range of hosts affected. The antigens chosen were apparently the ten most important serovars affecting domestic animals in the country (Joseph, 1979; Tan, 1970*a*). However, more appropriate serovars would be selected as antigens in future studies. As shown by Bahaman *et al.* (1984) and Hathaway & Little (1981), a more accurate prevalence would be achieved in using antigens in the MAT which are representative of the indigenous serovars.

Test procedure

The microscopic agglutination test performed was a modification of the method as described by Cole, Sulzer & Pursell (1973). In our laboratory each serum sample was initially diluted (1/10) with phosphate-buffered saline (PBS) in a test tube. Twenty-five microlitres of PBS were placed in each well of the microtitre plate (Grenier, Germany) and an equal volume of the diluted serum sample was placed in the first row (the 'A' row) of the plate. The diluted serum (now 1/20) was then serially diluted (twofold) from the 'A' row to the 'H' row using a hand-held microdiluter (Dynatech, Malaysia). When this was done, 25 μl of the antigen were added to each well. Thus each well would then contain 25 μl of the diluted serum sample and 25 μl of the antigen. For each serum sample tested there would be eight dilutions ranging from 1/40 to 1/5120 after the addition of the antigen. The plate was then incubated for 90 min at 37 °C before examining for evidence of agglutination. The test was read by transferring a drop from each well on to a glass microscope slide, using a multiple dipper designed by Ryan (1978). The drops were

examined by dry darkfield microscopy at a magnification of 200×. A positive reaction was regarded as one in which 50% or more of the antigen (live leptospires) were agglutinated. The titre endpoint was taken as the last well in which 50% or more agglutination was observed.

Choice of minimum titre

The minimum titres quoted by various workers (Kingscote, 1985; Mackintosh *et al.* 1980; Little *et al.* 1981; Sebek *et al.* 1978) have varied from 20 to 800 and often appear to have been selected arbitrarily without any additional support. Blackmore, Marshall & Ingram (1976) have indicated that titres can decline to very low levels within a period of several months, and the adoption of a higher titre as a cut-off point could give a false impression of the prevalence of the disease in a herd. There were instances where infected animals had low titres but were still excreting leptospires in their urine. However, Broom & McIntyre (1948) report that it is generally agreed that specific agglutination of leptospires by the serum does not occur in the absence of infection, present or past, and that agglutination even if present in low dilution is proof of such infection. In this study the minimum titre considered is 40. The choice of this low cut-off point is a compromise and appears to be more relevant to the disease situation. This cut-off point would consider infected animals with low titres but at the same time eliminate spontaneous or non-specific titres. Blackmore, Bahaman & Marshall (1982) in New Zealand have expressed the same opinion.

RESULTS

Of the 3377 serum samples tested, 862 (25·5%) had antibody titres of 40 or greater to one or more of the antigens employed in the microscopic agglutination test (MAT), indicating a high prevalence of leptospiral infection in domestic animals in West Malaysia. This overall prevalence (25·5%) is much higher than that reported by Wisseman *et al.* (1955) and Joseph (1979). Results from this present study record the presence of agglutinating antibodies to the ten leptospiral serovars tested (Table 1) and it must be emphasized here that the serological results obtained were only serogroup-specific. A bacteriological study is in progress to identify the actual serovars involved. Almost 16% of the domestic animals examined had evidence of infection to serovar *hardjo*. The next two serovars of importance were *pomona* and *tarassovi*. Titres to the other seven antigens were not very frequent, and prevalence of each serovar often did not exceed 1·5%. Thus the primary leptospiral infections seen in the domestic animals in West Malaysia were mainly due to serovars from the Sejroe, Pomona and Tarassovi serogroups (Table 1).

Cattle

The overall serological prevalence of leptospiral infection in the cattle examined in West Malaysia is 40·5% (558/1378). Thirty-four per cent of the cattle tested had evidence of infection with serovar *hardjo*. Infection with serovar *tarassovi* with a prevalence of 7·2% was next in importance, followed by *pomona* infection, which had a prevalence of 3·4% (Table 1). The prevalence of leptospiral infection in

Table 1. *Serological prevalence of leptospiral infection in domestic animals in West Malaysia*

Animal species	Cattle	Buffaloes	Goats	Sheep	Pigs	Overall
No. of sera examined...	1378	429	657	44	869	3377
No. of sera positive Leptospiral antigens						
hardjo	446	78	7	0	1	532
	(33·8)	(18·2)	(1·1)	—	(0·1)	(15·8)
icterohaemorrhagiae	14	3	0	0	29	46
	(1·0)	(0·7)	—	—	(3·3)	(1·4)
pomona	46	9	13	3	54	126
	(3·4)	(2·1)	(2·0)	(6·8)	(6·2)	(3·7)
canicola	15	0	3	0	12	29
	(1·1)	—	(6·8)	—	(1·4)	(0·9)
tarassovi	99	41	0	0	17	157
	(7·2)	(9·6)	—	—	(2·0)	(4·6)
australis	13	20	2	0	4	37
	(0·9)	(4·7)	(0·3)	—	(0·5)	(1·1)
grippotyphosa	0	0	0	0	5	5
	—	—	—	—	(0·6)	(0·1)
ballum	4	0	0	0	16	20
	(0·3)	—	—	—	(1·8)	(0·6)
pyrogenes	30	15	1	0	4	49
	(2·2)	(3·5)	(0·2)	—	(0·5)	(1·5)
celledoni	22	0	6	0	1	29
	(1·6)	—	(0·9)	—	(0·1)	(0·9)
Total	558	133	29	3	139	862
	(40·5)	(31·0)	(4·4)	(6·8)	(16·0)	(25·5)

cattle from individual farms ranged from 0 to 65%. Forty-one per cent of the farms studied had prevalence rates over 30%, whilst most 29% of the farms had over 50% prevalence. On the whole the prevalence of leptospiral infection in the farms is high (Table 2). One of the farms apparently had no evidence of leptospiral infection. Table 2 shows that the prevalence of leptospiral infection is significantly higher ($P = 0{\cdot}009$) in the semi-intensive farms compared to the smallholdings. This is probably due to the higher stocking rate (more animals in a defined area) resulting in greater ease of transmission of the infection in the herd (intraspecies transmission). Investigation into the distribution of leptospiral infection in the various breeds of cattle in this study does not show any distinct prevalence in any of the breeds, but on the whole, when comparing imported or temperate breeds with the local or indigenous breeds, there is a significantly higher ($P = 0{\cdot}00001$) prevalence of leptospiral infection in the imported breeds. This could possibly be due to the imported or temperate breeds being reared in large farms facilitating easy transmission, whereas the indigenous animals were usually kept in small numbers (fewer than 15 animals per herd) and often far apart.

Buffaloes

The prevalence of leptospiral infection in buffaloes is similar to that in cattle. The overall prevalence of leptospiral infection in buffaloes in West Malaysia is 31%, which is the second highest among the domestic animals. Serovar *hardjo* is the predominant serovar; it is responsible for 18·2% of the leptospiral infection in buffaloes, followed by serovars *tarassovi* (9·6%) and *australis* (4·7%). In buffaloes,

Table 2. *Serological prevalence of leptospiral infection under different management systems*

Farms/abattoirs	Place	Prevalence of positive sera		
		Cattle	Buffaloes	Goats
		Semi-intensive farms		
Institut Haiwan	Kliang	66/203	NC	0/151
R. Sembilih	S. Alam	55/79	ND	ND
UPM	Serdang	65/173	78/194	3/55
P. T. Haiwan	Pantai	43/91	ND	0/36
P. E. Haiwan	S. Siput	53/94	ND	0/48
P. T. Haiwan	U. Behrang	68/118	ND	ND
P. T. Haiwan	K. Bahru	22/100	ND	ND
P. T. Haiwan	B. Arang	18/44	ND	2/66
P. T. Haiwan	A. Hitam	52/80	ND	ND
MARDI	Taiping	0/59	ND	ND
	Bk Ridan	ND	50/196	ND
P. T. Haiwan	G. Mati	ND	ND	6/36
		442/1041	128/390	11/392
		Smallholders		
R. Sembilih	S. Alam	55/175	3/13	ND
Smallholders	Perlis	20/39	ND	ND
Smallholders	Tapah	0/5	ND	ND
Smallholders	S. Besi	18/32	ND	ND
R. Sembilih	A. Keroh	7/21	0/8	ND
Smallholders	Cheras	6/30	ND	ND
Smallholders	Ipoh	9/29	ND	0/12
R. Sembilih	Ipoh	1/6	ND	ND
Smallholders	Tobiar	ND	2/18	ND
Smallholders	P. Langkawi	ND	ND	5/25
Smallholders	Kuantan	ND	ND	5/50
Smallholders	Bachok	ND	ND	2/15
Smallholders	K. Bahru	ND	ND	1/16
Smallholders	Tumpat	ND	ND	5/20
Smallholders	Merang	ND	ND	2/25
R. Sembilih	P. Pinang	ND	ND	1/102
		116/337	5/39	18/265

australis infection is third in importance (Table 1), whilst in cattle it was serovar *pomona*. In buffaloes the prevalence of infection in individual farms ranges from 0 to 40% (Table 2). This is similar to the prevalence seen in individual cattle farms. High prevalence of infection is seen in the semi-intensive farms, and this could be attributed to the high stocking rate resulting in ease of transmission within the herd (intraspecies transmission). On comparing the prevalence of infection between the semi-intensive farms (there are only a few in the country) and the smallholdings, the probability of infection in semi-intensive farms is significantly higher ($P = 0{\cdot}01$) (Table 2). It is observed that the prevalence of infection is not significantly different ($P = 0{\cdot}06$) between the swamp buffaloes and the murrah buffaloes.

Goats and sheep

Leptospiral infection in goats and sheep appears to be sporadic in nature. The overall prevalence of infection in goats is 4·4%, whilst in sheep it is slightly higher at 6·8% (Table 1). The dominant serovar affecting both goats and sheep is serovar

Table 3. *Serological prevalence of leptospiral infection in pigs in Selangor, Malaysia*

Commercial farms and smallholdings	Feb. 1982	June 1983	Jan. 1984	Overall
No. of sera examined	310	215	344	869
hardjo	—	1	—	1
icterohaemorrhagiae	28	1	—	29
pomona	16	31	7	54
canicola	8	4	—	12
tarassovi	13	4	—	17
australis	3	—	1	4
grippotyphosa	5	—	—	5
ballum	8	—	8	16
pyrogenes	—	4	—	4
celledoni	—	1	—	1
No. of sera positive	81	42	16	139
Percentage of sera positive	26·1	19·5	4·7	16·0

pomona (Table 1). The prevalence of leptospiral infection in individual farms appears to be low. The highest prevalence is 25%, seen in one of the smallholdings. A number of the goat farms are serologically free of leptospiral infection. Comparing the farms according to the management system, leptospiral infection is significantly higher ($P = 0{\cdot}01$) in the smallholdings (Table 2). As mentioned earlier, the goats in the intensive farming were hardly let out to graze and therefore have less chance of coming in contact with leptospiral carriers or to an infected environment (pastures, water supply, etc.).

Pigs

Pigs are third in importance regarding leptospiral infection. The overall prevalence of infection (16·1%) is considered high, as most of the animals examined were porkers, not considered to be infected due to the protective presence of maternal immunity acquired through colostrum. Given time, a high prevalence of infection is expected. Serovar *pomona* with a prevalence of 6·2% was the most important serovar affecting the pigs in this study. Next in importance is serovar *icterohaemorrhagiae*, with a 3·3% prevalence (Table 1). The first group of 304 pigs examined in 1982 had agglutinating antibodies to *icterohaemorrhagiae* (9·2%), *pomona* (5·3%) and *tarassovi* (4·3%) (Table 3). The second examination involving 215 animals in 1983 showed no significant number of positive titres to serovar *icterohaemorrhagiae*. In a more recent study a low prevalence of leptospiral infection was observed in a group of 344 animals. Serovar *pomona* is seen as the important serovar affecting pigs in this study. *Pomona* infection was detected in all three groups of animals and appears to be endemic in the pig population in Selangor.

DISCUSSION

Leptospirosis caused by a variety of leptospiral serovars undoubtedly occurs in the domestic animals in Malaysia. Thirty-seven leptospiral serovars have been isolated in Malaysia (Alexander *et al.* 1957; Bahaman & Ibrahim, 1987; Gordon-Smith *et al.* 1961; McCrumb *et al.* 1957; Tan, 1970*a*), and with this large number

of serovars present a wide variety of clinical syndromes are to be expected in animals and humans in Malaysia. However, the mild anicteric forms of the disease are recognized as being more common than the clinically severe icteric forms (Danaraj, 1950; Tan, 1970*b*; Turner *et al.* 1959). Serological evidence of leptospiral infection in approximately 15% of the human population in the rural areas should not, therefore, be ignored (Tan, 1981) and warrants detailed investigation. There are certain unique features related to leptospirosis in domestic animals in Malaysia. Although there is a large number of leptospiral serovars present in the country, only three leptospiral serogroups appear to be of significance in the domestic animals as disclosed by this study. The three serogroups were Hardjo, Tarassovi and Pomona (Table 1).

Serovar *hardjo* infection has been shown to be endemic in the cattle population in Italy (Andreani, Tolari & Farina, 1983), New Zealand (Hellstrom, 1980), the United Kingdon (Ellis *et al.* 1985) and many other countries. This serovar has been incriminated as the major cause of leptospiral infection in cattle, causing abortions, infertility, weak progeny, mastitis and other economic losses (Ellis *et al.* 1982*a*, *b*; Hathaway, Little & Stevens, 1982). It has been shown that cattle are the maintenance host for this serovar (Hathaway *et al.* 1984*b*). The high serological prevalence of leptospiral infection reported by previous workers (Arunasalam, 1975; Leong & Maamor, 1975; Veterinary Research Institute Annual Reports, 1979–85), as well as from the findings of this present study, indicate that cattle in West Malaysia are the likely maintenance host for this serovar. Currently, there has not been any report of *hardjo* infection in animals in Malaysia other than in the ruminants. However, overseas reports have mentioned isolation of serovar *hardjo* from both man and pigs. Our results (Table 1) show that leptospirosis due to Sejroe serogroup is the most common in cattle and buffaloes in West Malaysia. The presence of Sejroe serogroup titres in almost half of the number of animals surveyed indicates that the distribution of infection is widespread. The high within-herd prevalence of Sejroe serogroup titres in cattle suggests a high frequency of intraspecies transmission within the herds. This may occur following the introduction of the infecting organism to the susceptible herd. On the other hand, the low within-herd prevalence of titres to Sejroe serogroup in the other animal species suggests that although sporadic infection in individual animals may be common, intraspecies transmission of the infecting serovar is limited.

No signs or symptoms of leptospiral infection were seen in the cattle in this study in spite of the high prevalence of serological reactors to serovar *hardjo*. At a glance, it appears that the pathogenicity of the serovar in cattle is low, but this is to be expected in an endemic situation. If cattle become infected early in life as often occurs in endemic situations, and immunity develops as a result, clinical symptoms will not occur. Thus the strains seen in Malaysia may not be of low virulence but may be influenced largely by management practices. In the United Kingdom *hardjo* infection has been associated with clinical syndromes such as abortion and agalactia (Ellis *et al.* 1982*a*). The difference between the cases in the United Kingdom and Malaysia could be in the strains of leptospires and/or the breeds of cattle involved. *Hardjo* infection in cattle in New Zealand is similar to that in Malaysia, where overt signs of disease are not a prominent feature (Dixon, 1983). Although cattle are not found in large numbers in Malaysia, they are widely

distributed throughout the country, and assuming that they are the maintenance host for serovar *hardjo* they would provide an extensive source of *hardjo* infection for other cattle as well as the other animal species, particularly buffaloes and goats.

One of the surveys that has been carried out to determine the extent of leptospirosis in buffaloes in Malaysia is that by Ryu (1969). He reported that almost 50% of the buffaloes examined had evidence of leptospiral infection. The Veterinary Research Institute Annual Reports (1979–85) also recorded a high prevalence of leptospiral infection. In contrast to the study by Ryu (1969), this present study revealed that 31% of the buffaloes examined had evidence of leptospiral infection and a significant percentage (18·2%) of the titres were to serovar *hardjo*. Agglutinating antibodies to serovar *tarassovi* were next in prevalence (9·6%), whilst infections to the other serovars appeared to be sporadic, as indicated by the low prevalence of significant titres. Thus, it was shown that serovars *hardjo* and *tarassovi* were the two serovars affecting the buffaloes in this study. Earlier reports (Gordon-Smith *et al.* 1961; Wisseman *et al.* 1955) in Malaysia showed much lower prevalence of leptospiral infection in buffaloes. In the Philippines, Carlos *et al.* (1970) demonstrated a high prevalence of leptospiral infection in the buffaloes. The positive reactors were mainly to serovars *tarassovi* and *sejroe*. McGuire & Myers (1957) found that 14% of the buffaloes in Egypt had leptospiral infection. They observed that there was a greater prevalence of infection in buffaloes than cattle in that country.

The low serological prevalence of leptospiral infection observed in goats and sheep in this study is not much different from those reported overseas (Chappell, Hanson & Garrigus, 1961; Ciuchini *et al.* 1980; Schollum & Blackmore, 1981; van Riel & van Riel, 1956). The low prevalence of infection could be due to the behaviour of the animals as well as management factors. The majority of the goats and sheep in Malaysia are reared in raised sheds, so that there tends to be minimal contact between the animals and their urine or the urine of other animal species. Urine of infected animals has been established as the primary source of leptospiral infection. Other factors accounting for this low prevalence are that goats and sheep are usually let out to graze late in the day, and that by nature these animals prefer dry environments, which are not conducive to the survival and spread of leptospires. There is also the possibility that goats and sheep are naturally more resistant than cattle to leptospiral infection. In this study, serovar *pomona* was shown to be the most important affecting sheep and goats. The prevalence of leptospiral infection in both species of domestic animals was rather low, indicating that the infection was sporadic. Most of the positive animals were from smallholdings where there is no proper management of the goats. In this system, the goats are let out to graze in any available pastures and the animals therefore could, by chance, have come in contact with some carriers or a contaminated environment. It is not evident from where the sheep and goats contracted the *pomona* infection. Overseas, pigs are known to be the maintenance host for serovar *pomona* (Hathaway, 1981; Hathaway *et al.* 1984*a*). The *pomona* infection seen in the goats and sheep in this study could possibly be of porcine origin.

The prevalence of infection in pigs seen in this present study is higher than that reported by Gordon-Smith *et al.* (1961). This is in spite of the majority of the

animals examined being porkers. A much higher prevalence would have been expected if the animals had been sows and boars. It is interesting to note that there was a steady decline in the prevalence of infection during the study period (from February 1982 to January 1984). The reasons for this decline have not been investigated. Vaccination of domestic animals against leptospirosis has not been implemented in Malaysia. However, there has been an increase in the number of intensive farms and subsequently, improved husbandry and management practices. It is observed that a majority of the large or commercial pig farms in Malaysia incorporate antibiotics in feeds as growth promoters, and this could possibly be one of the factors for the decline in infection. This present study disclosed the presence of agglutinating antibodies in pigs to all ten antigens used in the MAT. Five leptospiral serogroups appear to be significant; these are, in order of importance, Pomona, Icterohaemorrhagiae, Tarassovi, Ballum and Canicola. The serovar from Pomona serogroup, as in most countries, appears to be the most important serovar affecting pigs in this present study. The distribution of titres (the titres, although few, appear regularly in the three samplings) suggests that Pomona serogroup infection is endemic in the pig population in Selangor. Titres to the other antigens were very few in number (less than 4% prevalence) and appeared quite irregularly in the three samplings, suggestive of sporadic infection (Table 3).

Prior to the sampling, an abortion epidemic in which serovar *icterohaemorrhagiae* was implicated was reported in pigs in Selangor (Brandenburg & Too, 1981). Serovar *icterohaemorrhagiae* has been isolated from rats on many occasions (Fletcher, 1928; Gordon-Smith *et al.* 1961) and rats are now recognized as the maintenance host for this serovar. Many pig farms often had problems of rat infestation, and farms could easily become contaminated with leptospires through infected rat urine. In the first sampling (February 1982), serovar *icterohaemorrhagiae* was the predominant serovar affecting pigs sampled, but in the next two samplings it was observed to be insignificant (Table 3). One possibility is that the management could have controlled the rat infestation and eliminated the source of *icterohaemorrhagiae* infection. Joseph (1979) has also reported clinical manifestation of leptospirosis in pigs in Malaysia, but the causal serovars were not mentioned.

The significance of leptospiral infection in Malaysia is seen in the pig industry, where intensive farming is practised. Economic losses have been attributed to infertility and abortion epidemics (Brandenburg & Too, 1981; Joseph, 1979). With feed-lotting currently becoming popular in cattle farming, cattle are expected to play a bigger role than otherwise in the epidemiology of certain leptospiral infections. It is recognized that wild life, particularly rats, plays a major role in the dissemination of leptospirosis in Malaysia, and this is seen in the high prevalence of leptospiral infection in workers involved in plantations and forests, as well as soldiers on jungle operations. Rats, which are the maintenance host for most of the leptospiral serovars found in Malaysia, will be attracted to farms and houses where there is abundance of feed, and in the process the rats will disseminate leptospiral infection not only to farm animals but also to humans. The farm animals, in turn, will be an additional source of infection to farmers, the farm and its effluent.

With the advent of sophisticated and intensive pig and cattle farming in

Malaysia, a higher incidence of leptospiral infection will be seen not only in the domestic animals but also the people involved in this industry. This change might alter the epidemiology of leptospiral infection in Malaysia, which has been until now confined to very rural areas.

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