SERODIAGNOSIS OF LEPTOSPIROSIS IN DOMESTIC ANIMALS AND HUMANS

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ABSTRACT. A total of 3430 serum samples from various animal species and humans were tested using microscopic agglutination test (MAT) to determine the frequency of the important leptospiral serovars involved in animals and humans. The sera were screened against 14 serovars of pathogenic *Leptospira interogans* and 1 serovar of non-pathogenic *Leptospira biflexa*. Altogether, 441 (12.86%) of the tested serum samples were found to be positive serologically. Tested sera reacted to all 15 serovars used in this study. The most predominant serovar in cattle and sheep is *hardjo* (39.60% and 66.67%). However, in goat, buffalo and horse, the most frequent serovar detected is *hebdomadis* (30.00%, 32.58% and 57.14%). In dog, the most predominant serovar is *bataviae* (19.23%). In humans, the most predominant serovar is *cynopteri* (3.26%). Among all the samples tested, there were no positive samples from pig and cat. Domestic animals, rodents and pets can infect the environment or transmit the disease to human or other animals. This study showed that domestic animals could play a role in the epidemiology of leptospirosis and represents a threat to public health.

Keywords: Leptospira interrogans, microscopic agglutination test (MAT), serovars, zoonosis

INTRODUCTION

Leptospirosis is a global zoonotic bacterial disease of animals and humans with significant public health concern. This disease can infect various species of animals and humans and are thought to be the incidental hosts (Koteeswaran, 2006). It is caused by pathogenic spirochetes of the genus Leptospira belonging to the family Leptospiraceae (Angeliki *et al*., 2010). Over 200 pathogenic *Leptospira* serovars are known presently (El Jalii, 2008). Although most of leptospiral infections are subclinical, it may cause important economic losses to livestock farmers due to clinical signs of abortion, stillbirth, infertility, mastitis, weak, decrease milk production and may cause death depending on the virulence of the infecting serovar (Ellis, 1984). Death is accompanied by any combination of renal failure, liver failure and pulmonary haemorrhage (Bharti *et al*., 2003). Serovars are maintained in infected reservoir hosts (wild and domestic animals)

that serve as potential source of infection in human and other animals (Jamshidi *et al*., 2009).

The overall case fatality rate in human is 1% - 5%, which depends on the form of the disease whereby the elderly have a higher risk of getting infected (OIE, 2005). Humans become infected through direct contact with urine, blood and tissues of infected hosts. It also can be transmitted indirectly by ingestion of contaminated water or food, as well as direct contact with contaminated soil and water from the environment (Russ *et al*, 2003). The organisms enter the body through mucous membrane or abraded skin. In the environment, *Leptospira spp.* can remain up to several months under favourable condition. Epidemiological studies showed that, leptospirosis is more prevalent during wet season (Sekhar *et al*., 2000).

Information on leptospirosis from the previous study showed that, the serovar distribution varies in each country. It is important to determine the prevalence of serovars present in this country before deciding the appropriate control measure to be taken. Thus, the purpose of this study was to determine the frequency of the leptospiral serovars in various animal species and humans as detected by microscopic agglutination test (MAT) for control measures to be implemented in Malaysia.

MATERIAL & METHODS

Screening of serum of animals was conducted at the Serology Section, Veterinary Research Institute (VRI), Ipoh. Serological data from January 2010 to December 2010 were used in this study. Serum samples from various domestic animals (598 cattle, 359 buffalo, 959 goats, 507 sheep, 126 horse, 14 pig, 26 dog and 12 cat) and 829 sera from humans were submitted by the state veterinary services, pet clinics and government hospitals for evidence of agglutinating antibodies to leptospiral antigens tested. Altogether, 3430 sera were tested against 14 live antigens of *Leptospira interogans* : (*L. interrogans* serovar *australis, ballum, bataviae, canicola, cellodoni, cynopteri, djasiman, grippotyphosa, hebdomadis, hardjo, icterohaemorrhagiae, pomona, pyrogenes* and *tarassovi*). Besides, the sera were tested for antibodies against 1 live antigen of *Leptospira biflexa* serovar *patoc* as well. Sera were tested using microscopic agglutination test (MAT) as described by Cole *et al.* (1973). All sera were screened at a final dilution of 1:100 against these antigens. Results were considered positive when 50% or more agglutination was observed.

RESULT

Seropositivity analysis

Table 1 shows the distribution of seropositive leptospira reactors using MAT

among domestic animals and humans. Out of 3430 sera tested, 441 (12.87%) sera had a positive reaction against one or more serovars. These include samples from buffalo (35.10%), horse (30.16%), cattle (27.26%), human (9.77%), sheep (2.37%) and goat (0.83%). There were no positive samples from pig and cat.

Antibodies against more than one serovar was found in all species of animals which are positive for leptospirosis. A total of 41.72% from the seropositive samples showed serological reaction to more than one serovar (multiple reactors). The majority of the positive samples showed agglutination around 50% to 75% of leptospires at the test serum dilution of 1:100.

Seropositivity of different serovars

Seropositivity was seen in 15 serovars tested using MAT. In considering the positive samples, regardless of the host species, that is eitheranimals and humans; antibodies were most frequently observed to serovar *hebdomadis* (22.60%), *hardjo* (21.63%), *pomona* (18.71%), *tarassovi* (16.28%), followed by descending order to *icterohaemorrhagiae* (5.95%), *cellodoni* (3.40%), *cynopteri* (3.40%), *australis* (2.67%), *pyrogenes* (2.07%), *grippotyphosa* (1.22%), *canicola* (0.85%), *bataviae* (0.61%), *patoc* (0.36%), *ballum* (0.12%) and *djasiman* (0.12%). The frequency of infecting serovars in the domestic animals and humans are shown in Figure 1.

Table 2 shows the predominant serovar detected using MAT and the reaction of the serum against serovar tested. In cattle and sheep, the most predominant serovar was *hardjo* (39.60% and 66.67%). However, in goat, buffalo and horse, the predominant serovar detected was *hebdomadis* (30.00%, 32.58% and 57.14%). In dog, the predominant serovar

Table 1: Seropositive samples according to species tested using MAT

Species	No. of samples	Positive MAT	Negative MAT	Reactor rate $\left(\frac{Positive\ MAT}{Total\ no.of\ samples} \times 100\right)\%$
sheep	507	12	495	2.37%
goat	959	8	951	0.83%
buffalo	359	126	233	35.10%
cattle	598	163	435	27.26%
pig	14	0	14	0.00%
horse	126	38	88	30.16%
dog	26	13	13	50.00%
cat	12	0	12	0.00%
human	829	81	748	9.77%
TOTAL	**3430**	**441**	**2989**	**12.87%**

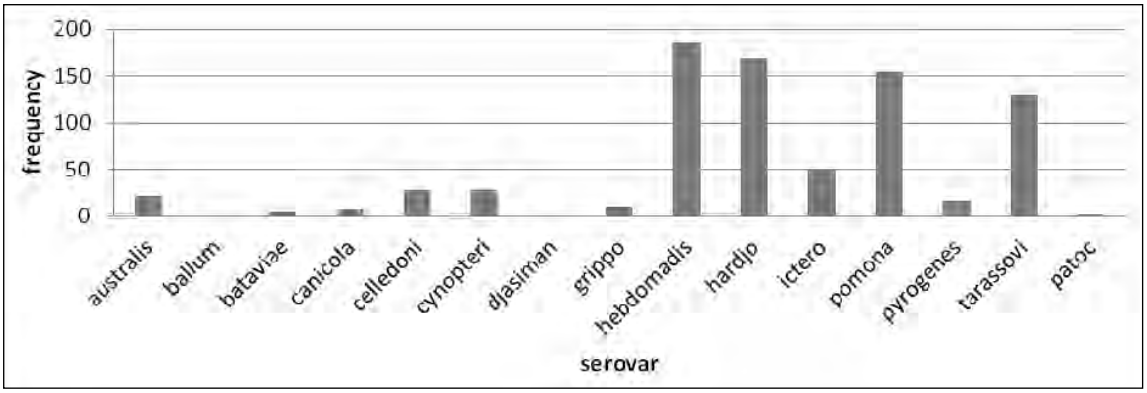

Figure 1: Frequency of leptospiral serovars in domestic animals and human.

Table 2: Predominant serovars detected using MAT

Animal	Reaction against serovar	1	2	3
Sheep	+ve to 2 serovars	*Hardjo* (66.67%)	*Tarassovi* (33.33%)	None
Goat	+ve to 6 serovars	*Hebdomadis* (30.00%)	*Australis* (25.00%)	*Hardjo* (20.00%)
Buffalo		*Hebdomadis* (32.58%)	*Pomona* (29.32%)	*Hardjo* (18.05%)
Cattle		*Hardjo* (39.60%)	*Tarassovi* (26.73%)	*Pomona* (14.36%)
Horse	+ve to 5 serovars	*Hebdomadis* (57.14%)	*Icterohaemorrhagiae* (28.57%)	*Pomona* (6.12%)
Dog	+ve to 12 serovars	*Bataviae* (19.23%)	*Australis* (15.38%)	*Pomona/Pyrogenes* (11.54%)
Human	+ve to 11 serovars	*Cynopteri* (24.11%)	*Cellodoni* (23.21%)	*Pyrogenes* (12.5%)

was *bataviae* (19.23%) and in human, the predominant serovar was *cynopteri* (24.11%).

DISCUSSION

Leptospirosis is caused by a variety of pathogenic leptospiral serovars. Most of the pathogenic serovars belong to *Leptospira interrogans,* while saprophytic *Leptospira biflexa* occur in the environment but of lesser significance in causing disease (Victoriano *et al.*, 2009). The reservoir hosts vary with the serovars and the geographical distribution. Thirty-seven serovars have been isolated from animals and humans in Malaysia (Bahaman *et al.*, 1987). The leptospiral serovars selected in this study are important serovars circulating in the country. Due to the variety of infecting serovars, a wide range of clinical syndromes of leptospirosis can be expected. However, most of the cases

occur subclinically or with mild clinical signs to self-limiting systemic illness.

The predominant serovar detected in cattle was serovar *hardjo* which is similar to previous studies on animal leptospirosis in Malaysia (El Jalii, 2008; Bahaman *et al*., 1987). It seems that serovar *hardjo is* well adapted and maintained in cattle (Joseph, 1979). Hajikolaei *et al*., (2007) reported that leptospirosis in sheep and goat are less frequent than in cattle, thus supporting the finding in this report which shows the lowest reactor rate in the small ruminant.

Leptospirosis is rare in cat (OIE, 2005), but a study by Jamshidi *et al*. (2009) stated that, there are few cases of leptospirosis in both household and stray cats. Growth promoters added with antibiotics in feed is a common practice in pig farming in Malaysia. This could be the reason of reduced cases in pig (Bahaman *et al*., 1987).

There was no data on clinical signs suggestive of leptospiral infection at the time of sampling in most animals (mainly in livestock) during this study. There was a case involving cats and dog, where leptospirosis was a part of the differential diagnosis due to clinical signs of fever and jaundice associated with kidney and liver failure.

Leptospirosis is known as an occupational disease where the high risk group include plantation workers, sewer maintenance staff, livestock farmers, abattoir workers, veterinarians and military personnel (Bharti *et al*., 2003). Report from OIE (2005), 8% to 29% of people who work with livestock have antibodies against *Leptospira spp.*. In this study, samples from human were obtained partly from veterinary staff, farmers, military staff, pet owners and patients from government hospitals. Samples from hospitals were from patients having fever complicated with old-age and kidney failure.

The environmental factors have shown to have effects on development of leptospiral infection in animals (Haji Hajikolaei *et al*., 2006). Many epidemiological studies noted that high prevalence of leptospirosis occur during wet season. Average temperature in Malaysia is between 21^{o}C to 32^{o}C with high humidity within 70% to 90% and copious rainfall (Malaysian Meteorological Department), thus the weather conditions in this country may favour the survival of pathogenic leptospires outside the host (Haji Hajikolaei *et al*., 2006). In majority of the samples, multiple reactors was detected which could be due to mixed serovar infection or cross-reactivity among serovars. The agglutination was observed within the test serum dilution of 1:100 indicating that leptospiral infection in Malaysia is endemic and mostly occurs in subclinical form. In endemic area where the animals become infected early in life, immunity will develop, thus no clinical signs can be observed. The difference in frequency of serovars in animals may depend on the susceptibility of the animal species to certain serovar.

CONCLUSION

Domestic animals may contribute and is the major source of infection in humans which act as important maintenance host for leptospirosis. Animals and humans in this country could be exposed to a variety of leptospiral serovars.. Control of the disease in domestic animals (livestock and pets) will reduce the risk in human population, but the existence of wildlife reservoirs (rodents) will contaminate the environment and may complicate the control strategy.

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