

Serovar diversity of *Leptospira* sp. infecting wild rodents in Sarawak, Malaysia

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Abstract. Leptospirosis is a zoonotic disease with global distribution and rodents, in particular rats, have been identified as the main reservoir host. A study was conducted to determine the prevalence of antibodies against *Leptospira* sp. in wild rodents caught in selected areas of Sibul, Sarikei and Kapit in Sarawak during the period of July 2011 to May 2014. In total, 241 sera samples were collected from rodents caught from these three administrative divisions in Sarawak. Ninety-eight rodents (40.7%) were positive with antibody titre $\geq 1:50$ by microscopic agglutination test (MAT) against 13 out of 20 common local leptospiral serovars tested. Sera of rodents caught in Sibul, Kapit and Sarikei divisions were positive at 43.9%, 37.5% and 36.4%, respectively. The top five serovars detected were: Autumnalis (25.5%), Tarassovi (23.5%), Bataviae (15.3%), Hebdomadis (8.2%) and Celledoni (7.2%). The main species of rodent positive for antibodies against *Leptospira* sp. were *Sundamys muelleri* (50.0%), *Rattus rattus* (37.5%), *Callociurus notatus* (35.6%) and *Rattus exulans* (32.6%). This study indicates that leptospiral antibodies are prevalent amongst wild rodents in central Sarawak, which could be translated as high leptospiral carriage. The close interaction that exists between the local community and the environment could potentially propagate the transmission of *Leptospira* sp. to human in these areas. This study also provided essential information about local circulating *Leptospira* serovars, which could be useful for eventual prevention measures in disease transmission.

INTRODUCTION

Leptospirosis is a zoonotic disease that affects both animals and human. The causative organism, *Leptospira* sp. is maintained in wild and domestic animals. Small mammals are the most important reservoirs, and rodents, especially rats are often implicated in the maintenance and spread of the infection to humans through direct or indirect transmission (Adler, 2015).

Leptospira sp. is maintained through chronic renal infection of the animal hosts and infections are generally acquired during infancy. These infected hosts are able to excrete live leptospires via their urine into

the environment throughout their life (Bharti *et al.*, 2003). Shed leptospires are able to survive in the environment, thus facilitating indirect transmission to humans (Haake & Levett, 2015, Tangkanakul *et al.*, 2000). In Malaysia, the wet and warm climate can sustain the survival of the shed leptospires for several days (Khairani *et al.*, 2004).

Rodent species such as *Rattus norvegicus*, *Rattus tiomanicus* and *Rattus rattus* have been identified as *Leptospira* sp. carriers in Malaysia (Mohamed Hassan *et al.*, 2010 & 2012, Benacer *et al.*, 2013). The prevalence of antibodies against *Leptospira* sp. in rodents and their serovar distribution varied widely and are influenced by many

factors such as the total numbers of rodents caught, geographic location of study area and the number and types of serovars tested (Cosson *et al.*, 2014). Several studies have been conducted on the diversity of *Leptospira* sp. in wild animal reservoirs in Sarawak, but these studies and the areas surveyed were rather limited (Thayaparan *et al.*, 2013, Pui *et al.*, 2017). Despite the endemicity of leptospirosis in Malaysia, limited research have been conducted on circulating serovars in animal reservoirs in Malaysia, particularly in Sarawak. This study aims to determine the seroprevalence of leptospirosis and the common circulating serovars in rodents caught in the Rejang basin of Sarawak.

MATERIALS AND METHODS

Sibu, Sarikei and Kapit administrative divisions form the central part of Sarawak and are served mainly by the Rejang river and its basin. The sample collections were conducted during the period of July 2011 till May 2014. The sites for rodent samplings were based on the seroprevalence study of human leptospirosis that reported a seroprevalence of at least 37.4% (Suut *et al.*, 2016). Rodent samplings were performed in areas close to human dwellings in eight locations. The locations are: a) Sibu – Bukit Aup, Kanowit, Tanjung Manis and Belawai; (b) Sarikei – Sarikei town (outskirt) and Bintangor; (c) Kapit – Song and Nanga Merit.

The trapping sites were located approximately 50 metres to 2 kilometres from the nearest human dwellings. At least 50 traps were set up at one trapping site and placed at about 10 meters apart from each other, both on the ground and on the trees alternately at both sides of the track. Each track was at least 500 metres long, but varied from one site to another as determined by the vegetation density and local terrain. At least four (4) trapping days were observed, and the traps were checked twice daily. Due to limited resources, no extra trapping days were added for the low capture numbers. Trapped rodents were collected, identified and photos of essential physical characteristics

were taken for species identification. Rodents' identification was conducted with the assistance from the staff of Zoology Department, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. Identification was also done based on the presence of essential characteristics according to Musser & Carleton (2005). The ethical approval for this study was obtained from Universiti Malaysia Sarawak Animal Ethics Committee (UNIMAS/AEC/R/F05/004).

Caught rodents' were euthanized using diethyl ether and blood samples were collected through cardiac puncture. The sera were kept at -20°C prior to analysis. Microscopic agglutination test (MAT) was performed against 20 live leptospires cultures prevalent in Malaysia and were obtained from the Institute of Medical Research (IMR) Culture Collection, Kuala Lumpur. The tested serovars were: Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Copenhageni, Djasiman, Grippothyposa, Hardjobovis, Hebdomadis, Icterohemorrhagiae, Javanica, Panama, Patoc, Pomona, Pyrogenes and IMR 175 (a local serovar), Shermani and Tarassovi. Two phases of MAT reaction were performed: a) qualitative MAT to identify the agglutinating serovar, and b) quantitative MAT for the determination of agglutination titre. The MAT reaction was read using the dark field microscopy by at least two researchers. Sera samples that exhibited antibody titre of $\geq 1:50$ were considered as positive, and two-fold dilution was done to determine its endpoint agglutinating titre. Serovar with the highest titre was recorded as the reacting serovar in samples that exhibited multiple serovar positivity. A titre of $\geq 1:50$ was accepted as positive in this study, as this cut-off titre is adopted in other similar studies in South East Asia and other endemic countries (Costa *et al.*, 2015; Loan *et al.*, 2015; Wangroongsarb *et al.*, 2002, Villanueva *et al.*, 2010). Furthermore, infected animals may demonstrate MAT titres below the recommended minimum significant titre of 100 (Hamond *et al.*, 2012; Otaka *et al.*, 2012).

RESULTS

A total of 241 rodents belonging to 10 species were caught during the study. Sibü recorded the highest number of caught rodents (64.3%), followed by Sarikei (22.0%) and Kapit (13.7%). *Sundamys muelleri* (Müller's Giant Sunda Rat) – 41.5% (100/241), *Callosciurus notatus* (Plantain Squirrel) – 19.1% (46/241), *Rattus exulans* (Pacific rat) – 18.7% (45/241) and *Rattus rattus* – 13.3% (32/241) were the main species caught during the sampling period. Other species and their geographical distributions are as shown in Table 1.

Ninety-eight rodents (40.7%) were positive for antibodies against leptospira. Rodents caught in Sibü showed the highest positivity (43.9%), followed by rodents in Sarikei (37.5%) and Kapit (36.4%). *Sundamys muelleri* (50.0%), *Rattus rattus* (37.5%), *Callosciurus notatus* (35.6%) and *Rattus exulans* (32.6%) were the main species that were positive for anti-leptospira antibodies (Table 1), whereas the numbers of other rodent species were too small to form any significant conclusions. *Sundamys muelleri* made up the highest proportion of rodents positive for agglutinating antibodies against *Leptospira* sp.; 51.7% & 38.5% in Sibü and Kapit, respectively, while in Sarikei, the

antibodies were mainly detected in *Rattus exulans* (38.9%).

Thirteen out of 20 tested serovars were detected in positive rodents (Table 2). Serovars Autumnalis (25.5%), Tarassovi (23.5%), Bataviae (15.3%), Hebdomadis (8.2%), Celledoni (7.2%) were the main five serovars detected. In Sibü, serovar Autumnalis predominated, followed by Tarassovi, Bataviae and Hebdomadis. In Sarikei, serovars Autumnalis and Bataviae were detected more frequently, and serovar Tarassovi was found to be more prevalent in Kapit. Thus, in general, the predominant serovar detected in rodents in the central part of Sarawak was Autumnalis. Agglutinating antibodies against serovars commonly associated with rats; Icterohaemorrhagiae (1%) and Copenhageni (2%) was detected less frequently in all areas. The highest MAT titres were observed against serovars Canicola (n=1; 1:800 dilution) and Tarassovi (n=2; 1:800 dilution).

DISCUSSION

The prevalence of agglutinating leptospira antibodies in wild rodents was 40.7%. Overall, the current estimate of leptospirosis infection

Table 1. Distribution of seropositive rodent species caught in study areas

Species	Study areas		
	Sibü	Sarikei	Kapit
	No. positive/Total (%)	No. positive/Total (%)	No. positive/Total (%)
<i>Sundamys muelleri</i>	44/85 (51.7)	1/2 (50.0)	5/13 (38.5)
<i>Callosciurus notatus</i>	10/28 (35.7)	3/11 (27.3)	2/7 (28.6)
<i>Rattus exulans</i>	2/9 (22.2)	14/36 (38.9)	–
<i>Rattus rattus</i>	8/21 (38.1)	0/3 (0)	4/8 (50.0)
<i>Rattus argentiventer</i>	1/7 (14.3)	–	–
<i>Niviventer cremoriventer</i>	–	0/1 (0)	0/2 (0)
<i>Rattus tiomanicus</i>	2/2 (100)	–	0/1 (0)
<i>Tupaia minor</i>	0	–	–
<i>Maxomys rajah</i>	–	–	1/2(50.0)
<i>Echinosorex gymmnurus</i>	1/1 (100)	–	–
Total Positive/Total (%)	68/155 (43.9)	18/53 (34.6)	12/33 (36.4)

Table 2. The distribution of *Leptospira* sp. serovars in seropositive rodents according to study areas

<i>Leptospira</i> sp.	Serovar	Study areas			Total no. (%)
		Sibu	Sarikei	Kapit	
		No. positive (%)	No. positive (%)	No. positive (%)	
<i>L. interrogans</i>	Autumnalis	17 (17.3)	7 (7.2)	1 (1.0)	25 (25.5)
	Bataviae	9 (9.2)	6 (6.1)	–	15 (15.3)
	Hebdomadis	7 (7.2)	–	1 (1.0)	8 (8.2)
	Pyrogenes	5 (5.1)	–	–	5 (5.1)
	Canicola	5 (5.1)	–	–	5 (5.1)
	Australis	4 (4.1)	–	–	4 (4.1)
	Copenhageni	2 (2.0)	–	–	2 (2.0)
	Panama	–	1 (1.0)	–	1 (1.0)
	Icterohaemorrhagiae	–	–	1(1.0)	1 (1.0)
<i>L. borgpetersenii</i>	Ballum	–	1 (1.0)	–	1 (1.0)
	Tarassovi	15 (15.3)	–	8 (8.2)	23 (23.5)
<i>L. santarosai</i>	Shermani	–	–	1(1.0)	1 (1.0)
<i>L. weilii</i>	Celledoni	4 (4.1)	3 (3.1)	–	7 (7.2)
Total (%)		68 (28.3)	18 (18.4)	12 (12.2)	98 (100)

in wild rodents tested in this study was higher from the seroprevalence in other studies conducted in Malaysia and the neighbouring countries (Pui *et al.*, 2015; Cosson *et al.*, 2014; Loan *et al.*, 2015; Benacer *et al.*, 2013; Thayaparan *et al.*, 2013; Latifah *et al.*, 2012; Mohamed Hassan *et al.*, 2010). Nevertheless, the highest seroprevalence of leptospira in rats in Malaysia was reported in a landmark study by Smith *et al.*, 1961, in which 92% of black rats tested were positive for anti-leptospira antibodies. In this study, rodents caught in Sibru recorded the highest prevalence of anti-leptospira antibodies. This observation could be attributed to the higher numbers of rodents captured at the sampling sites. The high prevalence of leptospira antibodies in the studied rodents indicated a high leptospiral carriage, and highlighted the endemicity of the *Leptospira* sp. in rodents in Sarawak.

It was observed that *Sundamys muelleri*, *Rattus rattus*, *Callociurus notatus* and *Rattus exulans* demonstrated the highest positivity for leptospira antibodies in this study. This observation differed slightly from the previously reported local studies, where

R. tiomanicus (Mohamed Hassan *et al.*, 2010), *R. rattus* and *R. norvegicus* (Benacer *et al.*, 2013) predominated. In the Mekong delta of Vietnam, *Rattus norvegicus* and *Bandicota indica* were among the main vectors implicated (Loan *et al.*, 2015). The apparent disparity in the species of rodents reported in these studies is probably attributed to the geography of the areas being studied. In the current study, most of the study locations are rural villages situated along the Rejang river and fringed by forests. This could have accounted for the high frequency of *S. muelleri*, *R. rattus*, *R. exulans* and *C. notatus* in this study, as these species thrive in such habitats (Wells *et al.*, 2008). It would be best that rodents' species identification to be done on-site by the expert. However, due to limited resources and also as it was not feasible to bring all the rodents' carcasses back to the laboratory, photographic representation of important features of caught rodents were used for identification in some study areas. In order to avoid misidentification of the rodents' species, further clarifications were sought from the experts.

Multiple serovar positivity were observed in seropositive rodents in this study, which might be due to serological cross-reactions. The cross reactions could be due to infection with more than one serovar at any particular time, or two or more past infections. However, in this study, only serovar with the highest end-point agglutination titre was reported, and considered as the current infecting serovar. Thirteen (13) serovars were detected in positive rodents, and serovars Autumnalis, Tarassovi, Bataviae, Hebdomadis, and Celledoni accounted for the majority of the reactive sera. Serovars identified in this study varied from those reported previously (Mohamed Hassan *et al.*, 2010; Benacer *et al.*, 2013; Thayaparan *et al.*, 2014; Loan *et al.*, 2015; Villanueva *et al.*, 2010). The variation in leptospiral serovar reported in this study underlined the diversity of circulating serovar in various geographical locations within Malaysia, and other countries.

The predominance of serovars Autumnalis and Bataviae in this study corroborated the recognized serovar host association in rats and small rodents (Faine *et al.*, 1999). However, serovars Icterohaemorrhagiae and Copenhageni were detected less frequently in this study. This could be attributed to the species of rodents or the limited number of rodent individuals caught. The detection of serovar Tarassovi, which is usually associated with pigs (Kemenes & Siveges, 1976), might indicate the role of rodents as incidental host. Unfortunately, this assumption cannot be further investigated due to the economic importance of pigs to the local communities. The diverse serovars found in the studied rodents could also reflect the ability of the organism to continually adapt to new hosts or vice versa. This could contribute to the complexity of interaction dynamics between the organisms, host, surrounding environment and humans as observed previously by other researchers (Hartskeerl *et al.*, 2011).

Seven of the tested serovars were not detected in this study, and the non-reactivity of the rodents' sera could signify the absence of these serovars. However, the limited number and species of animals studied, and

limited number of serovars tested could have contributed to this observation. A robust selection of study areas, sampling sites and more varied animal species sampling should be attempted to address this limitation. On the other hand, the existence of untested serovars in the studied rodents could not be ruled out, as faunal diversity in Sarawak is diverse and might harbour untested serovar/s. Positive isolation of *Leptospira* sp. through culture and species identification by molecular methods is worth pursuing in order to provide essential epidemiological information regarding *Leptospira* sp. in animals in Sarawak.

The high prevalence of antibodies against *Leptospira* sp. reflected the endemicity of leptospira in rodents in these areas. The rodents could potentially act as reservoirs and drive the transmission to human through direct or indirect contact. Sarawak has the fifth highest incidence rate of human leptospirosis in Malaysia (Benacer *et al.*, 2016), and data from the Sarawak Health Department showed that 27.6% of human leptospirosis cases in Sarawak (year 2009-2016) were from Kapit, Sibuan and Sarikei divisions (unpublished data). It is possible that the human leptospirosis in these areas is associated with the rodents' leptospiral carriage. The rapid development currently experienced in these study areas through dam construction, opening of vast plantation, as well as agrarian environment and other subsistence activities performed by the local communities could have facilitated the interaction between human and the rodents' host. However, this association needs to be further examined and should be confirmed through strain typing of isolates obtained from human and rodents. It is important that effective preventive measures in disease transmission and increased awareness regarding leptospirosis is communicated to these communities to curtail *Leptospira* transmission.

This study emphasizes the high prevalence of *Leptospira* in wild rodents in Sarawak, and provides preliminary information on the epidemiological distribution of *Leptospira* serovars and their associated animal hosts. Further study in

other reservoir species from other parts of Sarawak, followed by leptospiral isolation and subsequent strain typing should be explored to provide a wider perspective of *Leptospira* epidemiology in reservoir animals, and would be of useful in the eventual prevention of human leptospirosis.

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