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Serological and molecular detection of *Leptospira* spp. from small wild mammals captured in Sarawak, Malaysia

S Thayaparan[1*], Ian D Robertson[1] and Mohd Tajuddin Abdullah[2]

[1]College of Veterinary Medicine, School of Veterinary and Life Sciences, Murdoch University, 6150 Murdoch, Western Australia.
[2]Centre for Kenyir Ecosystems Research, Institute for Kenyir Research, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
Email: thayaan@yahoo.com

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ABSTRACT

Aims: Leptospirosis is endemic to tropical regions of the world and is re-emerging as a new danger to public health in Southeast Asia, including Malaysia. The purpose of this particular study was to determine the common leptospiral serovars present in small wild mammals living around wildlife reserves and disturbed forest habitats and human communities.
Methodology and results: The samples of blood and kidneys of small rodents, bats and squirrels were analyzed. Antibodies to different serovars of leptospires were detected in 73 of 155 wild small mammals captured (47.0%: 95% CI 39.0-55.3%). The seroprevalence for rats (57.9%; 95% CI 44.1-70.9) was slightly higher than that for squirrels (42.9%; 95% CI 24.5-62.8) and bats (40%; 95% CI 28.5-52.4). Seropositive animals were detected in all 5 localities sampled. Antibodies to serovar Lepto 175 Sarawak were detected in 30 (24.7%) rats, 11 (9.0%) squirrels and 27 (52.9%) bats. Of 155 kidney samples from individual animals only 17 were positive for *Leptospira* on a molecular study (10.97%, 95% CI 6.5-17). The majority of the positive results were from plantain squirrels (53%; 95% CI 27.8, 77), Müller's rat (35%; 95% CI 14.2, 61.7) and brown spiny rats (12%; 95% CI 1.5, 36.4).
Conclusion, significance and impact of study: This particular study should generate concerns and lead to the health authorities expanding disease control measures in the region as there are significant levels of human activity at all five locations where the animals were sampled. The pathogenesis of serovar Lepto 175 Sarawak also needs to be monitored closely, considering its similarities to the pathogenic *Leptospira wolffii*.

Keywords: Leptospirosis, zoonotic disease, bats, rodents, wildlife

INTRODUCTION

Leptospirosis is endemic to tropical regions of the world and is re-emerging as a new threat to public health in Southeast Asia, including Malaysia. Although studies have been conducted in Malaysia for more than 70 years (Hanson, 1982), the threat leptospirosis poses is still not well understood. Malaysian jungles are home to numerous species of wildlife and peri-domestic animals. These include bats, squirrels and rats, as well as primates. Bats and rats are known to harbour leptospiral serovars in their bodies and pass them to other species including humans (Roth, 1964; Faine *et al.*, 1999; Richardson and Gauthier, 2003; Matthias *et al.*, 2005; Vashi *et al.*, 2010).

Rodents have long been associated with leptospirosis as reservoir or maintenance hosts and verminous rodents are considered a key source for the distribution of leptospires in an urban setting (Faine *et al.*, 1999; Slack *et al.*, 2006; Priya *et al.*, 2007; Harris, 2009; Lau *et al.*, 2010a; Lau *et al.*, 2010b; Tulsiani, 2010; Costa *et al.*, 2014). Rats have been shown to be carriers of leptospires throughout the world and are important reservoirs of infection for animals and humans (Roth, 1964; Priya *et al.*, 2007). Recently bats have also been implicated in the spread of leptospirosis to humans (Mortimer, 2005; Vashi *et al.*, 2010).

With deforestation becoming more commonplace in many tropical environments, including Sarawak, Malaysia, there is increased likelihood of contact between humans and wildlife due to the disturbance of natural habitats. The increasing popularity of eco-tourism raises another risk for the disease. Such tourism brings visitors to Malaysia close to nature and native wildlife, some of which may be reservoir hosts for leptospirosis, as well as potentially interfering with an already-fragile ecosystem.

The purpose of this study was to determine the common leptospiral serovars present in small wild mammals living around wildlife reserves, disturbed forest

***Corresponding author**

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habitats and human communities around Kuching, Sarawak, Malaysia.

MATERIALS AND METHODS

Study and sampling sites

The sampling of animals was undertaken in the wider ecological ranges of Kuching, and Kota Samarahan area, including Universiti Malaysia Sarawak (UNIMAS) area, Matang Wildlife Centre, Kubah National Park, Bako National Park, Wind and Fairy Cave Reserves and Mount Singai Conservation Area (Figure 1). Sampling sites were chosen to cover different ecological environments (e.g. disturbed habitats and secondary forests located around human settlements) to gain an accurate representation of the possible transmission of leptospires. All locations sampled in the current study were within approximately 30 kilometres of Kuching, the capital city of Sarawak. A common trend in most of the human leptospirosis outbreaks in the Kuching region have been exposure to wildlife and contact with water contaminated by leptospires (Thayaparan *et al.*, 2013a). Bako National Park, Matang-Kubah Wildlife Centre, Mount Singai and the Wind and Fairy caves are known to contain several wildlife species (Hall *et al.*, 2004; Khan *et al.*, 2007; Thayaparan *et al.*, 2013a) that may be potentially accountable for transmitting leptospires (Abdullah, 2011). UNIMAS is located in an area where the natural environment has been disturbed, similar to other areas of Sarawak. Certain parts of the UNIMAS campus are also known to be waterlogged and have patches of forested environment, increasing the possibility of exposure of students at the campus to leptospires.

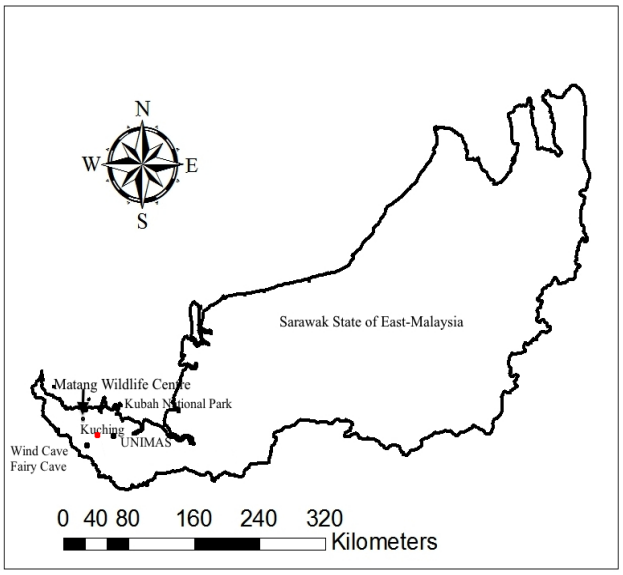

Figure 1: Sampling sites of small wildlife in Kuching and Kota Samarahan, Sarawak.

Animal capturing and ethical aspects

During the investigation 155 individual small mammals were captured and sampled. The species sampled in this study included any small mammals (rats, squirrels, tree shrew and bats) were captured in the selected areas over a one-year period from January 2011 to March 2012.

The rats, squirrels and tree shrews were trapped using cage traps (Baeumler and Brunner, 1988). A maximum of 50 cage traps were set around the border of a wildlife area and human settlement, with the intention of trapping small mammals at one sampling time. A mixture of one-part raisins and two parts peanut butter with rolled oats, banana and dry fish was used as bait and placed in each trap (Hickie and Harrison, 1930; Wood, 1984). Traps were opened from 6 am and checked once the afternoon and following morning. Any by-catch was immediately released at the site of trapping. If pregnant or lactating small mammals were captured these were also released immediately at the captured site. Any trapped rats, squirrels and tree shrews were collected and taken to the UNIMAS laboratory for processing. The cage was covered with cloth and kept in the animal house until processing. Each small mammal was housed in individually ventilated cages. During the period of transportation small mammals were fed sunflower seeds, cereals, cooked corn kernels and cheese and provided with water *ad libitum*.

The rodents were anaesthetised using Ketamine + Xylazine (50–75 mg/kg + 10 mg/kg IP) (Karwacki *et al.*, 2001). Once the rats were anaesthetised 1-3 mL of blood was collected via cardiac puncture using a 25G needle and a 5 mL syringe. After blood collection, the rats were euthanased with barbiturate (sodium pentobarbitone at 150-200 mg/kg) via either an intracardiac or intravenous (IV) injection. After euthanasia the animals were necropsied and kidney samples collected. Blood samples were left at room temperature for 30 min to clot and were then centrifuged at 3,000 RPM for 1 min. The serum was removed and stored at –20 °C until testing in the laboratory.

All the bats were wild-caught either using mist nets and harp traps and terrestrial small mammals using cage traps. Mist net is a lightweight net that was placed in the flight path of the bats, for example across walking or animal trails, over streams, adjacent to fruiting trees and at the mouths of caves. Bats that were caught in the nets were carefully removed from the net to ensure their delicate wings were not injured. Harp traps were also set across trails or over small streams. Three harp traps and five mist nets were placed along bat flight paths for a maximum of six hours between 6 pm and 11 pm. Nets were checked every 30 min and any bats caught were removed from the traps. After removal each bat was placed in an individual cloth bag. The cloth bags were hung and tied in a handmade wicker basket. The basket was covered with a cloth and placed in a safe quiet place to minimise disturbance. If any birds were trapped these were removed and released immediately.

Bats or small mammals were anaesthetised to reduce the stress of handling and to minimise the risk of the handler

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being bitten. Each animal was anaesthetized with an intramuscular injection of ketamine (6–7 mg/kg) and medetomidine (60–70 µg/kg) (Plowright *et al.*, 2008). A blood sample (1 to 3 mL) was collected by cardiac puncture from each bat using a 5 mL syringe and a 25G needle. Prior to collection the hair was sterilized with cotton wool dipped in 70% alcohol. The bats were placed in a fabric pouch and placed in a plastic airtight container. Cotton balls were thoroughly saturated with isoflurane in the barrel of a six ml syringe (after removing the plunger). The syringe barrel containing the isoflurane saturated cotton balls were then placed into the container and the lid closed. Bats were usually anaesthetised within seconds and death was confirmed by auscultation with a stethoscope. Kidney samples were removed for culturing and molecular analysis. Carcasses were stored in 99% ethanol for future use by UNIMAS students and kept as museum voucher specimens following Abdullah *et al.* (2010). All personnel who handled bats had been vaccinated against rabies and all samples were treated as potentially infectious.

Trapping of wildlife was approved by the Murdoch University Animal Ethics Committee (Animal ethics No: W2376/10) and Sarawak Forestry Department, Sarawak Malaysia (Permit No: NCCD.907.4.4 (V)-235).

Diagnostic methods

Microscopic Agglutination Test (MAT)

The Microscopic Agglutination Test (MAT) was performed according to Faine (1982) to check for *Leptospira*-specific antibodies to 17 serovars commonly found in Malaysia: Australis, Autumnalis, Bataviae, Canicola, Icterohaemorrhagiae, Celledoni, Grippotyphosa, Javanica, Pomona, Pyrogenes, Hardjo, Tarassovi, Patoc, Djasiman, Lai, Copenhegeni, Lepto 175 Sarawak. The preliminary results for Lepto 175 Sarawak have been presented previously (Thayaparan *et al.*, 2013b). Serum positive at a titre of 1:50 was further titrated until 1:1600.

Molecular analysis

Culture and isolation and preparation of genomic DNA from kidney samples

Kidney samples from all animals were cultured for leptospires. A cross-section of the cortex of one kidney was removed using a sterile scalpel and this section was cut into small pieces and inoculated into two EMJH and two Fletcher media tubes for culture. The remaining part was deposited in 100% pure PCR grade alcohol for future PCR work. The tubes with the inoculated material were incubated at 30 °C and checked twice weekly under dark field microscopy for 12 weeks. If there was no growth after 12 weeks, or if any fungal growth was detected, the specimen was discarded. Approximately 30 mg of kidney was placed into a sterile Petri dish. Using a sterile scalpel and a needle the tissue was dissected into small pieces. The tissue was then transferred into a micro-centrifuge tube and mixed with 50 µL protein kinase K (Qiagen) and 200 µL lysis buffer and incubated at 55 °C overnight or until the tissue was completely digested.

Analysis of PCR products

Cultures were prepared for DNA isolation by centrifugation as described previously (Thaipadungpanit *et al.*, 2007), followed by genomic DNA extraction using a High Pure Viral Nucleic Acid kit (Roche, Germany). Amplification of the 16S rRNA was performed as described by others (Slack *et al.*, 2006; Thaipadungpanit *et al.*, 2007; Slack *et al.*, 2008;) with the following modifications: PCR amplification was performed in 25 µL volumes containing 1x *Taq* PCR buffer, 2.0 mM $MgCl_2$, 200 uM dNTPs, 10.0 pmol forward primer (5'- GTT TGA TCC TGG CTC AG 3') and 10.0 pmol reverse primer (5'-CCG CAC CTT CCG ATAC-3') (Thaipadungpanit *et al.*, 2007), 1U *Taq* Polymerase (Fermatas, Cat#-EP0405), 2.5 µL template DNA and nuclease free water to make up the final volume of 25 µL.

Polymerase chain reaction

The DNA was amplified by using the following thermal-cycling profile: 95 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 58 °C for 30 sec and 72 °C for 1 min, and a final extension for 7 min at 72 °C. DNA sequencing was performed using the Big Dye Terminator (BDT) sequencing kit version 3.1 (Applied Biosystems) using the original primer as described previously (Thaipadungpanit *et al.*, 2007). The PCR products were confirmed by agarose gel electrophoresis (1.5% w/v) of 5 µL PCR products for 45 min at 116V.

DNA sequencing

The cycle-sequencing products were purified by using sodium acetate-alcohol precipitation as per the manufacturer's instructions (Applied Biosystems) and the purified products were forwarded to the First Base Laboratories Sdn Bhd, DNA sequencing facility, Selangor, Malaysia, for capillary electrophoresis using an ABI 3130XL instrument.

Analysis of sequence results

The sequences were assembled and trimmed to a minimum of two contiguous sequences using Bio Edit software (Invitrogen). Sequences from identified strains and representative 16S rRNA (1331 bp) gene sequences from members of the genus Leptospira were aligned with CLUSTAL W (Thompson *et al.*, 1997). By using MEGA 5 (Tamura *et al.*, 2011), distances of aligned 16S rRNA gene sequences were estimated by the Jukes-Cantor method (Jukes and Cantor, 1969), bootstrapped 1000 times and the tree topology was determined by the neighbour-joining method (Slack *et al.*, 2006; Slack *et al.*, 2008; Slack *et al.*, 2009). The final phylogenetic tree was rooted by using *Turneriella parva* serovar Parva strain H as an out-group and bootstrap values (1000) were displayed as percentages (Thaipadungpanit *et al.*, 2007).

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RESULTS

In total 155 animals were sampled including 70 bats, 57 rodents, 20 squirrels, and eight treeshrew. The rats trapped were Müller's rat (*Sundamys muelleri*) (n=29), ricefield rat (*Rattus argentiventer*) (n=20), brown spiny rat (*Maxomys rajah*) (n=7), and whitehead's rat (*Maxomys whiteheadi*) (n=1). All squirrels were either Low's squirrels (*Sundasciurus lowii*) (n=1) or plantain squirrels (*Callosciurus notatus*) (n=19) and captured treeshrew (*Tupaia tana*) (n=8). The bats netted and trapped were dusky fruit bats (*Penthetor lucasi*) (n=20), short-nosed fruit bats (*Cynopterus brachyotis*) (n=22), spotted-winged fruit bats (*Balionycteris maculata*) (n=13), fawn roundleaf bats (*Hipposideros cervinus*) (n=5), Bornean horseshoe bats (*Rhinolophus borneensis*) (n=2), bicolored roundleaf bats (*Hipposideros bicolor*) (n=2), hollow-faced bat (*Nycteris tragata*) (n=1), intermediate horseshoe bat (*Rhinolophus affinis*) (n=1), dusky roundleaf bat (*Hipposideros ater*) (n=1), lesser woolly horseshoe bat (*Rhinolophus sedulus*) (n=1), papillose woolly bat (*Kerivoula papillosa*) (n=1) and dayak roundleaf bat (*Hipposideros dyacorum*) (n=1). The largest number of animals was caught in the locality of UNIMAS (40) followed by Wind Cave and Fairy Cave areas (36), Bako National park (31), Matang and Kubah (30) and Mount Singai (18) (Tables 1 and 2).

Table 1: Species, location and MAT results for animals other than bats caught.

Species	UNIMAS	Mt. Singai	Wind & Fairy	Matang & Kubah	Bako	Total	%	MAT (+)	% Positive (95% CI)
Sundamys muelleri	10	6	3	4	6	29	34.1	20	68.9 (42.9, 84.7)
Rattus argentiventer	6	4	4	2	4	20	23.5	9	45.0 (23.1, 68.5)
Maxomys rajah	0	1	4	2	0	7	8.2	4	57.1 (18.4, 90.1)
Maxomys whiteheadi	0	0	1	0	0	1	1.2	0	0.0 (0, 97.5)
Sundasciurus lowii	0	0	0	1	0	1	1.2	1	100.0 (2.5, 100)
Callosciurus notatus	4	2	4	3	6	19	22.4	10	52.6 (28.9, 75.6)
Tupaia tana	0	0	1	4	3	8	9.4	1	12.5 (0.3, 52.7)
Total	20	13	17	16	19	85	100.0	45	52.9 (41.8, 63.9)
%	23.5	15.3	20.0	18.8	22.4	100.0			

Table 2: Species, location and MAT results for bats caught.

Species	UNIMAS	Mt. Singai	Wind & Fairy	Matang & Kubah	Bako	Total	%	MAT (+)	% Positive (95% CI)
Penthetor lucasi	5	2	11	0	2	20	28.5	12	60.0 (36.1, 80.9)
Cynopterus brachotis	10	3	0	4	5	22	31.4	9	40.9 (20.7, 63.6)
Balionycteris maculate	5	0	0	4	4	13	18.5	3	23.1 (5.0, 53.8)
Hipposideros cervinus	0	0	2	3	0	5	7.1	2	40.0 (5.3, 85.3)
Other bat species	0	0	6	3	1	10	14.2	2	20.0 (2.5, 55.6)
Total	20	5	19	14	12	70	100.0	28	40.0 (28.5, 52.4)
%	23.5	15.3	20.0	18.8	22.4	100			

Serological and descriptive analysis

The seroprevalence for rats (57.9%; 95% CI 44.1-70.9) was slightly higher than that for squirrels (42.9%; 95% CI 24.5-62.8) and bats (40%; 95% CI 28.5-52.4), however these differences were not significant (χ^2= 4.28; df=2; P=0.117).

The highest seroprevalence was found in Müller's rat (68.9%; 95% CI 42.9-84.7) followed by brown spiny rat (57.1%; 95% CI 18.4-90.1), plantain squirrel (52.6%; 95%

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CI 28.9-75.6) and ricefield rats (45%; 95% CI 23.1-68.5). The highest seroprevalence in bats was observed in the dusky fruit bat (60%; 95% CI 36.1-80.9), followed by the short-nosed fruit bat (40.9%; 95% CI 20.7-63.6) and spotted-winged fruit bat (23.1%; 95% CI 5.0-53.8) (Tables 1 and 2). There was no significant difference in the seroprevalence between the different animal species (χ^2= 24.9; df=18; P=0.126).

Some seropositive animals were detected in all of the localities sampled. The highest prevalence was found at Mount Singai (64.7%; 95%CI 38.3-85.8) followed by Matang and Kubah (56.7%; 95%CI 37.4-74.5), Wind Cave and Fairy cave (47.4%; 95%CI 30.4-64.5), followed by Bako National Park (45.2%; 95%CI 27.3-64.0) and UNIMAS area (35%; 95%CI 20.6-51.7) (Table 3). There was no significant difference in the seroprevalence between the five sampling location (χ^2= 4.28; df=4; P=0.117), however significantly more seropositive animals were detected at Mount Singai than at UNIMAS (OR 3.4; 95% CI 1.04, 11.17).

For the analysis of locality UNIMAS was selected as the referent to ensure that all OR were greater than 1. Small mammals from Mount Singai were 3.4 times (95% CI 1.04-11.17) more likely to have leptospiral antibodies than animals captured from the UNIMAS area. Animals from Matang and Kubah were 2.4 times (95% CI 0.92, 6.42) more likely to have antibodies than those from the UNIMAS area (not significant). Wind Cave & Fairy Cave and Bako National Park had odd ratios of 1.7 and 1.5 respectively (95% CI 0.66, 4.18; 0.59, 4.0). There was little difference between the risk of animals from UNIMAS and those from Matang and Bako being seropositive (95% confidence intervals including the value 1.0) (Table 3).

Table 3: Seroprevalence to leptospires in small mammals according to their locality.

Locality	No of seropositive	Seroprevalence (95% CI)	OR (95% CI)
UNIMAS	14	35.0 (20.6, 51.7)	1.0
Mt. Singai	11	64.7 (38.3, 85.8)	3.4 (1.04, 11.17)
Wind & Fairy Caves	17	47.2 (30.4, 64.5)	1.7 (0.66, 4.18)
Matang & Kubah	17	56.7 (37.4, 74.5)	2.4 (0.92, 6.42)
Bako NP	14	45.2 (27.3, 64.0)	1.5 (0.59, 4.0)

Table 4: Seroprevalence to leptospires in small mammals and their odd ratios.

Animals	MAT Positive	Seroprevalence (95% CI)	OR (95% CI)
Rats	33	57.9 (44.1, 70.9)	1.0
Squirrels & Treeshrew	12	42.9 (24.5, 62.8)	0.55 (0.24, 0.99)
Bats	28	40.0 (28.5, 52.4)	0.48 (0.22, 1.36)

Among the Rodentia (rats), Scandentia (Squirrel, treeshrew) and Chiroptera (bats), rats were selected as the referent animal group. The odds of disease in Scandetia and Chiroptera compared to Rodentia were 0.55 (95%CI 0.24, 0.99) and 0.48 (95% CI 0.22, 1.36), indicating these animals were less likely to be seropositive than rats; although the Chiroptera result was not significant (Table 4).

Antibodies to 10 different serovars were detected in rodents and six different serovars in bats. Antibodies to serovar Lepto 175 Sarawak were detected in 30 (24.7%) rats, 11 (9.0%) squirrels and 27 (52.9%) bats. Antibodies to serovar Icterohaemorrhagiae were detected in 20 (16.5 %) rodentia and scandetia; sv. Australis in 13 (10.6 %) blood samples and sv. Autumnalis in 12 (9.6 %) samples. Serovars Australis and Lai were detected in one dusky fruit bat and one short-nosed fruit bat respectively. Antibodies to serovar Pyrogenes were detected in 10 (19.6%) samples from dusky fruit bats and short-nosed fruit bats. The antibody titres for seropositive animals varied from 1:50 to 1:800. More animals had a serum dilution of 1:100 (n=65) than other dilutions (1:50 in 20 serum samples; 1:200 in 30 serum samples and 1:400 in 8 samples, while only one squirrel had a dilution of 1: 800) (Table 5).

Culture and molecular analysis

None of the culture sample maintained in the lab produced positive results and majority of the specimens were contaminated and after four weeks all the samples were discarded. Out of 155 kidney samples from individual animals, only 17 were positive for *Leptospira* on the molecular study (10.97%, 95% CI 6.5, 17). The majority of the positive results were from plantain squirrels (53%; 95% CI 27.8, 77), Müller's rat (35%; 95% CI 14.2, 61.7) and brown spiny rats (12%; 95% CI 1.5, 36.4). No other animals were positive on the PCR (Table 6).

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Table 5: Titres of antibodies to different serovars found in small mammals.

Serovar	Titres					
	Positive	1:50	1:100	1:200	1:400	1:800
Lepto 175 Sarawak	68	23	27	12	5	1
Australis	15	3	5	4	3	0
Automnalis	12	2	6	4	0	0
Icterohaemorrhagiae	21	5	13	3	0	0
Javanica	5	1	3	1	0	0
Pyrogenes	10	8	2	0	0	0
Patoc	25	8	14	3	0	0
Copenhageni	6	0	4	2	0	0
Pomoma	5	2	2	1	0	0
Lai	5	4	1	0	0	0
Total/%	172/100	56/32.5	77/44.7	30/17.4	8/4.6	1/0.6

Table 6: PCR results from the kidneys of small mammals and their potential PCR using Neighbouring method.

Place	Species	PCR	Serovar
Mt. Singai	*Callosciurus notatus*	Positive	*L. pomoma*
	Sundamys muelleri	Positive	*L. icterohaemorrhagiae*
UNIMAS	*Sundamys muelleri*	Positive	*L. lai*
Wind Cave & Fairy Caves	*Callosciurus notatus*	Positive	*L. pomoma*
	Callosciurus notatus	Positive	*L. pomoma*
	Maxomys rajah	Positive	*L. icterohaemorrhagiae*
	Maxomys rajah	Positive	*L. icterohaemorrhagiae*
	Sundamys muelleri	Positive	*L. lai*
	Callosciurus notatus	Positive	*L. pomoma*
	Sundamys muelleri	Positive	*L. icterohaemorrhagiae*
Matang & Kubah	*Sundamys muelleri*	Positive	*L. icterohaemorrhagiae*
	Callosciurus notatus	Positive	*L. pomoma*
Bako National Park	*Callosciurus notatus*	Positive	*L. pomoma*
	Callosciurus notatus	Positive	*L. pomoma*
	Callosciurus notatus	Positive	*L. pomoma*
	Sundamys muelleri	Positive	*L. icterohaemorrhagiae*
	Callosciurus notatus	Positive	*L. pomoma*

DISCUSSION

The results indicate that small mammals from Mount Singai were 3.4 times more likely to have leptospiral antibodies than animals from the UNIMAS area. These findings highlight potential risk for acquiring leptospirosis to villagers living around Mount Singai and eco-tourists and recreationists visiting the forested mountain. The studies undertaken indicated a greater proportion of rats were seropositive than bats, squirrels and tree shrews, highlighting the importance of these species as carriers or reservoirs of leptospires.

Deforestation, which is becoming commonplace across Malaysia (Aiken and Leigh, 1992; Jomo *et al.*, 2004; Abdullah, 2011), can promote the emergence of

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infectious diseases, such as leptospirosis, by placing humans into contact with novel reservoirs or infectious agents (Arief, 2013; Matthias *et al.*, 2005). Bats respond to the destruction of their habitat at the level of populations and communities, making their spatial and temporal dynamics particularly sensitive to anthropogenic activity (Calisher *et al.*, 2006). Matthias *et al.* (2005) detected Leptospira serovar Icterohaemorrhagiae from one bat in the Peruvian Amazon region and proposed a rodent-bat cycle of infection. (Vashi *et al.*, 2010) added further support to the growing awareness of the role played by bats in the transmission of this pathogen to humans and other animals.

Forests in Southeast Asia, including Malaysia, are characterized by flowering and fruit production, substantially contributing to the abundance of rodents (Nakagawa *et al.*, 2007). A ricefield rat captured by Mohamed-Hassan *et al.* (2012) at a military training camp in West Malaysia was identified as a carrier of leptospires. Rats are also reservoirs of a number of other parasites and infectious pathogens, including the agent of plague, Yersinia pestis (Zahedi *et al.*, 1984).

Studies have shown that rodents and bats are reservoirs for leptospirosis (Matthias *et al.*, 2005; Vashi *et al.*, 2010). In the current study the positive PCR result from kidney samples are considered to be indicative of a carrier status of this pathogen in squirrels and rodents. In contrast animals positive on the MAT are not necessarily carriers as Leptospira-specific antibodies can be detected in convalescent sera and positivity to an MAT indicates past or current infection but not necessarily renal shedding (Faine *et al.*, 1999). In the current study, serovar Pomona was most prevalent among the plantain squirrels, being detected in kidneys collected from animals from four out of the five regions sampled. Müller's rats were identified as carriers of both serovar Lai and Icterohaemorrhagiae, with the latter also found in brown spiny rats. Many small mammals also displayed high levels of antibodies to the newly discovered strain sv Lepto 175 Sarawak, with plantain squirrels and Müller's rats being more commonly affected with this serovar. This Lepto 175 Sarawak was also found in a large number of dusky fruit bats tested. At present serovar Lepto 175 Sarawak is best considered an intermediate strain, as its lethal capacity is unknown.

According to (Thayaparan *et al.*, 2013b) Lepto 175 was shown to have a close similarity to *Leptospira wolffii* group, which has been isolated from Thailand, Iran and India (Zakeri *et al.*, 2010; Balamurugan *et al.*, 2013). Antibodies to serovar Icterohaemorrhagiae, a well-known pathogen implicated in many fatal cases of leptospirosis in humans, was the fourth-most common serovar detected after Australis and Autumnalis. Pyrogenes seemed to be more common in bats while Autumnalis was found mainly in rodents. Rice field rats tested positive for at least two known pathogenic strains.

An epidemic of leptospirosis has recently been reported in Malaysian Borneo, with Sarawak recording a nearly four-fold increase in the number of cases in 2011 as compared to 2010. A joint study by UNIMAS and the state health department in 2011 in the Rejang basin found that 31% of humans sampled were seropositive for leptospirosis and infection was associated with farming and/or water activities (Suut *et al.*, 2011).

CONCLUSIONS AND RECOMMENDATIONS

This particular study has generate concerns that should lead to the health authorities to expand their disease control measures as there are significant levels of human activity at all five locations where the animals were sampled. Visitors and eco-tourists should be advised to protect themselves and avoid direct contact with contaminated soil or water. Local nearby villagers who are workers at the Universiti Malaysia Sarawak and Universiti Teknologi MARA in Kota Samarahan should be screened to avoid major health risks among the students and academic staff. Wherever possible, pest control measures should be implemented to contain the rat population, particularly considering that these rodents appear to be the main source of infection in these locations.

Wildlife that is undergoing rehabilitation at captive facilities should be screened for Leptospira periodically before their eventual re-introduction to the natural environment. This helps prevent them from transmitting the disease to other species in the wild or to humans involved in their release.

The pathogenesis of serovar Lepto 175 Sarawak also needs to be monitored closely considering its similarities to *Leptopsira wolffii*. The latter has been isolated from both humans and animals by scientists in Thailand, Iran and India. It remains to be seen if serovar Lepto 175 Sarawak will be as virulent as *Leptospira wolffii* and hence extra vigilance is required and the Malaysian health authorities need to implement disease control measures to prevent the occurrence of a potential epidemic.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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