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RESEARCH ARTICLE
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Leptospiral agglutinins in captive and free ranging non-human primates in Sarawak, Malaysia

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Abstract

Aim: The proposed study was carried out to determine the extent of exposure to leptospirosis in non-human primates.

Materials and Methods: Trapping of non-human primates was carried out opportunistically around the Bako National Park and the Matang Wildlife Center in the vicinity of human settlements and tourism areas of Sarawak. Blood samples were obtained from the saphenous vein to determine the presence of antibodies by the Microscopic Agglutination Test (MAT) to 17 serovars of *Leptospira* commonly found in Malaysia.

Results: This study reports the screening of twelve primates (eight captive and four free ranging) for leptospirosis. Eight of the 12 monkeys (66.6%; 95% CI 34.9-90.1) reacted against one or two serovars of *Leptospira* (Lai and *Leptospira* Lepto175). The serovar Lai is considered pathogenic for different mammals, including humans. *Leptospira* Lepto 175 has been identified as an intermediate strain and further studies are being undertaken on this serovar.

Conclusion: These results are important as primates may act as reservoirs of *Leptospira* spp. for humans, which may potentially affect tourism (economic loss), conservation efforts and public health.

Keywords: leptospirosis, MAT, non-human primates, seroprevalence, wildlife, zoonotic disease.

Introduction

Leptospira have been detected from wildlife in many countries, however their role as reservoirs is still poorly understood [1-3]. Leptospirosis can result in economic losses in domesticated animals and has the potential to be an important zoonotic disease of humans [4]. Leptospires were first isolated from rats in 1917 and it is widely acknowledged that rodents are a key source of infection for humans [5]. However, recently Australian and Peruvian researchers have reported that bats can also carry pathogenic *Leptospira*, [1-2, 6], although their role as carriers is not fully understood. Other wildlife, including primates, can also act as potential carriers of these pathogens [7-10]. However to date there has been little research conducted on free ranging wildlife. Leptospirosis in wildlife can affect biodiversity, human and livestock health, animal welfare and consequently the national economy [4].

Recently leptospirosis has been recognised as a re-emerging public health problem in Malaysia [11]. At present Malaysian wildlife disease surveillance is poorly coordinated and emerging zoonotic infectious diseases represent a growing threat. Tourism is a major contributor to the economy of Malaysia with 24.6 million tourists visiting the country annually. It has been estimated that approximately one million tourists are involved in eco-tourism activities and this group is particularly at increased risk of exposure to infectious diseases [12-13].

In recent years outbreaks of leptospirosis in Malaysia have been documented around the wildlife reserves and parks resulting in confirmation of a high number of confirmed cases and associated mortalities. Wildlife tourism is an important source of revenue in Malaysia, particularly in the state of Sarawak and leptospirosis has the potential to impact on this. The current research reports on the carriage of *Leptospira* by opportunistically sampled non-human primates in Sarawak.

Materials and Methods

Ethical approval: All procedures were performed with the approval of the Animal Ethics Committee of the Murdoch University (W2376/10) and Sarawak Forestry cooperation (NCCD.907.4.4 (V)-235).

Study area: Trapping of monkeys was carried out around Bako National Park and Matang Wildlife Centre. Bako National Park is located 37 km from Kuching, Sarawak, East Malaysia (Figure-1). It is Sarawak's oldest national park, covering an area of 2,727 hectares and is located at the tip of the Muara Tebas Peninsula [14]. Although it is one of the smallest national parks in Sarawak, it contains almost most types of vegetation found in Borneo along with long-tailed macaques (*Macaca fascicularis*), silver-leaf monkeys (*Trachypithecus cristatus*), proboscis monkey (*Nasalis*

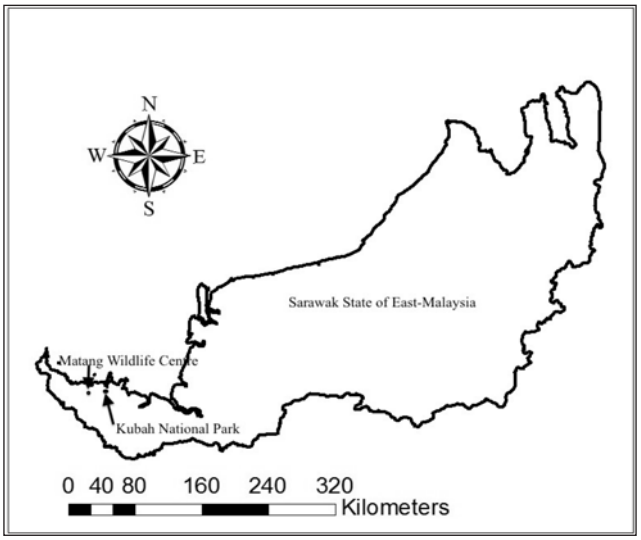

Figure-1: Location of research area in the Sarawak state of East-Malaysia

Table-1: MAT results obtained from sera of 12 primates in Sarawak.

Common name	Species	Place	*L.* Lepto175*	*L.* Lai
Short-tailed macaque	*Macaca nemestrina*	Matang (C)	800	400
Short-tailed macaque	*Macaca nemestrina*	Matang (C)	400	200
Short-tailed macaque	*Macaca nemestrina*	Matang (C)	400	<50
Proboscis monkey	*Nasalis larvatus*	Bako (F)	<50	<50
Proboscis monkey	*Nasalis larvatus*	Bako (F)	<50	<50
Proboscis monkey	*Nasalis larvatus*	Bako (F)	200	100
Bornean gibbon	*Hylobates muelleri*	Matang (C)	400	200
Bornean gibbon	*Hylobetes muelleri*	Matang (C)	200	<50
Orangutan	*Pongo pygmaeus*	Matang (C)	200	<50
Orangutan	*Pongo pygmaeus*	Matang (C)	<50	<50
Long-tailed macaque	*Macaca fascicularis*	Matang (C)	<50	<50
Silvered langur	*Trachypithecus cristatus*	Bako (F)	400	<50

(C): Captive animal; (F): Free ranging animals

larvatus), common water monitors lizards (*Varanus salvator*), plantain squirrels (*Collosciurus notatus*), wild boar (*Sus scrofa*) and mouse deer (*Tragulus kanchil*). The major attraction of this park is the presence of the proboscis monkey [14].

The Matang Wildlife Centre is situated at the western corner of the Kubah National Park in Sarawak [14], East Malaysia, and covers around 180 hectares of lowland forest. It is dedicated to education, research, conservation and recreational activities [14]. In this park several species of confiscated wildlife (long tailed macaques, pig/short-tailed macaques - *Macaca nemestrina*, Bornean gibbons - *Hylobates muelleri*, orangutans - *Pongo pygmaeus* and sun bears - *Helarctos malayanus*) are available for public viewing.

Sampling methods: In this study eight captive primates from Matang (three pig/short-tailed macaques, two Bornean gibbons, a long tailed macaque and two orangutans), and four free ranging primates from Bako (one silver-leaf monkey and three proboscis monkeys) were tested for the presence of leptospiral antibodies. Animals were tranquilised with Zoletil (5mg/kg; 100mg/ml). Five ml of blood was collected from the saphenous vein into a plain tube from each animal. The tubes were maintained at room temperature for 15 minutes and then centrifuged at 15,000 RPM for 5 minutes. The serum was then separated and stored at -20°C until it was analysed.

Microscopic agglutination test (MAT): MAT was performed according to Faine [15] to check for *Leptospira*-specific antibodies to 17 serovars (Australis, Autumnalis, Bataviae, Canicola, Celledoni, Copenhegeni, Djasiman, Grippotyphosa, Hardjo, Ictero haemorrhagiae, Javanica, Lai, Lepto 175 (Sarawak), Patoc, Pomona, Pyrogenes and Tarassovi). The preliminary results for *Leptospira*, Lepto 175 have been presented previously [16]. Test sera were diluted to 1:50 and then serially diluted two fold in phosphate buffered saline, to obtain dilutions of 1:100 to 1:1600. Sera were considered to be positive if the titre was 1:100 by the MAT.

Results

Antibodies to leptospires were detected in 8 (66.6%; 95% CI 34.9-90.1) of the 12 animals sampled (Table-2). Of the eight captive primates six were seropositive (75%; 95%CI 34.9-96.8). Two of the four free ranging primates also were seropositive (50%; 95% CI 6.8-93.2).

The antibody titres for seropositive animals varied from 1:100 to 1:800. More animals had a serum dilution of 1:200 (n = 5) and 1:400 (n=5). One captive short-tailed macaque had a titre of 1:800 to Lepto 175 (Sarawak) (Table-1).

Discussion

Positive antibodies to leptospires were detected in captive primates from the Matang Wildlife Centre. These infections may have arisen from poor sanitation or ineffective rodent control and adopting programs to improve these would likely minimise the spread of leptospires to other animals, including humans. Specifically the highest antibody titre (1:800) was detected against *L*. Lepto 175 (Sarawak) in a short-tailed macaque. This single high antibody titre may indicate active infection [10], however confirmation of its significance requires testing of serially collected samples.

In Bako National Park several short and long-tailed macaques and wild boars roam freely on the ground eating leftover food and garbage. The two species of macaque have the potential to transmit leptospires to proboscis and silver-leaf monkeys because these later species have an arboreal lifestyle. Besides contact with rats, ingestion of water contaminated with leptospires could be a potential source of infection as silver-leaf and proboscis monkeys drink from freshwater streams and rivers [17]. Proboscis monkeys are also competent swimmers [18-19] and are known to have the most aquatic lifestyle of primates rarely ranging more than one kilometre from water sources.

In this study a positive titre in a proboscis monkey was reported for possibly the first time in Malaysia. Information on Lepto 175 is deficient although it would appear to be endemic in Sarawak, although its pathogenicity has yet to be confirmed [16]. The serovars has close genetic similarities with wolffii, [16] which has been isolated from humans and wildlife species, including non-human primates, from Thailand, India, Iran and Sabah [10, 20-21]. Serovar Lai is pathogenic and is a major cause of zoonotic spread to humans involved in ecotourism adventure activities associated with water [22]. Studies by Kilbourn *et al.* [10] on free-ranging orangutans in Sabah found a high prevalence of antibodies against serovars *gryppotyphosa* and *autumnalis*, as well as the novel strain wolffii. Experimental infection of marmoset monkeys (*Callithrix jacchus*) with serovar Copenhageni resulted in the presence of histological evidence of kidney damage [23].

According to the results of previous researchers, natural infection of monkeys by leptospires is an unusual event, however it has been observed in serological surveys or under exceptional conditions in captivity [23]. Lilenbaum *et al.* [24] detected anti-Leptospira agglutinins in *Leontopithecus rosalia* (lion tamarins) in Rio de Janeiro, Brazil, even though these animals showed no clinical signs or prior history of disease. Romero *et al.* [25] reported the potential transmission of leptospires from zoo animals, in particular neotropical monkeys, to staff in Colombian zoos.

Conclusion

From this study, evidence of exposure of silver-leaf and proboscis monkeys to leptospires was found and there is an urgent need to undertake more investigations on free ranging non-human primates in Malaysia to understand their role in the transmission of leptospires. Although leptospires are endemic in Sarawak this is the first reported evidence of infection in non-human primates. The possibility of non-human primates acting as reservoir hosts for leptospires needs further investigation.

Authors' contributions

ST carried out the study and drafted the article. IDR and MTA participated in the scientific discussion, ST assisted in conducting MAT. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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