Borneo J. Resour. Sci. Tech. (2013) 2(2): 71-74

SHORT COMMUNICATION

Serological Prevalence of Leptospiral Infection in Wildlife in Sarawak, Malaysia

SIVA THAYAPARAN*[1], IAN ROBERTSON[1], FAIRUZ AMRAAN[2], LELA SU'UT[3] & MOHD TAJUDDIN ABDULLAH[4]

[1]School of Veterinary Medicine and Biomedical Science, Faculty of Health Science, Murdoch University, 6150 Murdoch, Western Australia; [2]Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia; [3]Lot 77, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Jalan Tun Ahmad Zaidi Adruce, 93150, Kuching, Sarawak, Malaysia; [4]Department of Zoology, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia

ABSTRACT

Leptospirosis is a zoonotic disease caused by pathogenic leptospiral bacteria, which are transmitted directly or indirectly from animals to humans or animal to animal. The first phase of this proposed study was carried out to determine the extent of exposure to leptospirosis in wild mammals surrounded by human settlements around wildlife or tourism area (Wind Cave, Fairy Cave, Bako National Park and Matang Wildlife Center). This study reports an incident of leptospirosis among primates (three captive and two free ranging), rats, bats, squirrels and mongoose around Kuching, Sarawak area, which has been screened for Leptospirosis. Blood samples were obtained to determine the presence of antibodies through the microscopic agglutination test (MAT) using eighteen serovars of *Leptospira* commonly found in Malaysia as antigens. It was observed that four out of the five monkeys (80%), rats (9/4) (44%), bats (20/5) (20.8%), squirrels 4/4 (100%) and mongoose (1) (100%) reacted against one or more serovars of *Leptospira*. In this study antibody of five serovars of *Leptospira interrrogans* Copenheni*, Leptospira interrrogans* Lai, *Leptospira interrrogans* Pomona, *Leptospira interrrogans* Pyrogenes, Lepto 175* were detected. Serovars Copenhegeni, Lai, Pomona and Pyrogenes were considered pathogenic for different mammals including human beings. No information about serovars lepto 175 and further studies going on. This is providing information on the possible zoonotic importance of mammalian species in maintaining this disease in Sarawak. The transmission of leptospires in rats reported several incidents and between primates, bats, squirrels, mongoose and human is not reported elsewhere but this could create new reservoir and transmission routes and may affect the tourism, conservation effort and public health.

Keywords: Leptospirosis, wildlife, mammals, Sararawak, Borneo

Leptospirosis can affect both humans and animals throughout the world resulting in morbidity and mortality (Russ *et al*. 2003; WHO Headquarters, Geneva, 2006). The important epidemiological feature of leptospirosis in domestic animals and wildlife can lead to economic loss and potentially spread to the human communities.

Leptospirosis can be transmitted in human by direct contact with infected blood, tissues, organs or urine of infected hosts or through indirect contact with contaminated formites, soil, mud, fresh water, vegetation and food stuffs or working in places infested with rodents (Terpstra 2003; Zavitsanou & Babatsikou 2008). Transmission can also occur via the direct penetration of the leptospires through the conjunctiva or surface epithelium (Russ *et al*. 2003). The role of rats as a source of human infection was discovered in 1917 (Levett 2001) and subsequently some researchers have identified that flying foxes can carry pathogenic leptospires in Australia (Cox *et al*. 2005; Smythe *et al*. 2002). The bacteria can cause polymorphic disease conditions in wild, domestic animals and in human (Terpstra 2003). However, to date there has been little research on the role of wildlife in outbreak throughout the world. Due to the current significant levels of reforestation occurring and the involvement of humans in the jungle, there is the potential for exposure of humans to new serovars of leptospires.

Trapping of monkeys, rats, bats, squirrels and mongoose were carried out around Wind

*Corresponding authors: *tabdulla@frst.unimas.my; thayaan@yahoo.com*

Cave, Fairy Cave, Bako National Park and Matang Wildlife Center. Rats, squirrels and bats were trapped alive, anaesthetized and blood extracted by cardiac puncture. Serum samples were kept at -20°C until perform the Microscopic Agglutination Test (MAT). To screen the status of *Leptospira*, three captive primates (*Macaca nemestrina, Hylobates muelleri, Macaca fascicularis*) and two free ranging primates (*Presbytis cristata, Nasalis larvatus*) have chosen for this study. 5 ml of blood collected in plain tube for the serum. After 15 min plain tube centrifuged at 15,000 RPM for 5 minutes and serum separated and stored under -20°C for MAT. The mongoose caught accidently in cage trap included into this screening and collected 5 ml blood from cephalic vein and serum separated and stored under -20°C before screen for leptospirosis antibodies.

The Microscopic Agglutination Test was performed according to Faine (1982) to check for *Leptospira*-specific antibodies from non-human primates, rats, bats, squirrels and mongoose. Serum was tested against 18 serovars using the following serovars: Australis, Autumnalis, Bataviae, Canicola, Copenhageni, Celledoni, Shermani, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Patoc, Pomona, Pyrogenes, Sejroe, Lai and Lepto 175 (Sarawak) commonly found in Malaysia. Serum positive at titre 1:50 was further titrated until 1:1600. Sera were considered to be positive if the titre was ≥ 1:50 by MAT.

A total of five primates, nine rats, 20 bats, four squirrels, and one mongoose were caught during the first phase of sample collection around wildlife area. Four out of the five monkeys (80%) *Macaca nemestrina, Hylobates muelleri*, *Presbytis cristata, Nasalis larvatus* showed the positive titre and *Macaca fascicularis* did not show any titre. Rats (9/4) (44%), bats (20/5) (20.8%), squirrels (*Callosciurus notatus*) (4/4) (100%) and mongoose (*Herpestes brachyurus*) (1) (100%), were positive for leptospiral antibodies from their serum. From the positive cases 72% were showed the positive titer with Lepto 175, 33% with Lai, 17% with Pomona, 11% with Copenhageni and 11% with Pyrogenes. Detailed information about antibodies indentified in different species shown below.

Table 1. Findings of antibodies of different leptospira serovars in particular species.

Order/Species	sv.lep175*.	sv.copen.	sv.lai.	sv.pom.	sv.pyro.
Macaca nemestrina	+	-	+	-	-
Hylobates muelleri	+	-	-	-	-
Trachypithecus cristatus	+	-	-	-	-
Nasalis larvatus	+	-	+	-	-
Sundamys muelleri	+	-	+	-	-
Ratus argentiventer	-	+	-	-	-
Cynopterus brachyotis	+	-	-	-	-
Penthetor lucasi	+	-	-	-	+
Nycteris tragata	-	-	+	-	-
Hipposideros cervinus	+	-	-	-	-
Callosciurus notatus	+	-	-	+	-
Herpestes brachyurus	+	-	+	-	+

Sv.copen.- copenhegeni, sv.lai.- lai, sv.pom.- pomona, sv.pyro.- pyrogenes, sv.lep175.- lepto175. *Leptospirosis Lepto 175 (Sarawak) strain was isolated from Sarawak environmental source and no further information on this strain and further studies is going on in Institute for Medical research (IMR) Kuala Lumpur.

Malaysia is endemic to *Leptospira* (Sejvar *et al.* 2003). In recent decade Malaysia is becoming famous for Eco-tourism and many tourists from various countries are visiting for Eco-challenge activities and wildlife tourism. Screening the wildlife around the wildlife area clearly showing there is a source of infection around those areas and it could cause direct transmission to humans or it could transmit from animals to human. Rats are well known

carrier of leptospirosis and spread among other animals and human. But bats, squirrels, mongoose and monkeys are not known about their carrier status and need more studies to explore their carrier status. A positive result on proboscis monkey is alarming the situation and its first time reported this case in Malaysia. The information on Lepto 175 (Sarawak) is unknown and its endemic in Sarawak Malaysia. The status of its pathogenic or not is not known and further studies is going on by Institute for Medical Research Kuala Lumpur, but the serovars Lai is pathogenic and its major cause of zoonotic spread from adventure activities, such as rafting, cannoning, and swimming in river (Sejvar *et al*. 2003). Past history of the human cases such as in 1984 there were two teams of cave explorers from British confirmed with *Leptospira* infection after their return from Mulu cave, Sarawak (Mortimer 2005). Again in 1985 another two British tourists confirmed with *Leptospira* after their return from Sarawak. After the 2000 Eco-challenge in Sabah, several participants from USA, UK, Australia and New Zealand are confirmed with *Leptospira* infection (Sejvar *et al*. 2003). This all incidents clearly indicating that *Leptospira* infection might be going to affect the tourism industry in future. It is best to find out the source of infection and take the preventive measures from *Leptospira* infection before getting any major outbreak.

ACKNOWLEDGEMENTS

The authors would like to thank Murdoch University and SOS rhino (US) to provide the financial support to conduct the research in Sarawak Malaysia. Especially thankful for Sarawak Forestry cooperation for providing permit (Permit No: NCCD.907.4.4 (V)-235) to capture rats and bats; the primate blood samples were collected using the Proboscis Monkey Genome Project lead by M.T. Abdullah. Thanks for the masters and undergraduate students from the Department of Zoology, Universiti Malaysia Sarawak, for helping to capture small mammals and identification. Special thanks go to staffs from Institute for Medical Research (IMR), Kuala Lumpur for technical support. This research is conducted according to the Murdoch Animal ethics (W2376/10).

REFERENCES

Antony, S.J. (1996). Leptospirosis–an emerging pathogen in travel medicine: a review of its clinical manifestations and management. *Journal of Travel Medicine*, 3(2): 113-118.

Cox, T.E., Smythe, L.D., & Leung, L.K.P. (2005). Flying foxes as carriers of pathogenic Leptospira species. *Journal of Wildlife Diseases*, 41(4): 753.

El Jalii, I.M. & Bahaman, A.R. (2004). A review of human leptospirosis in Malaysia. *Tropical Biomedicine*, 21(2): 113.

Faine, S. (1982). Guidelines for the control of Leptospirosis, World Health Organization, Geneva. WHO offset Publication No. 67.

Informal Consultation on Global Burden of Leptospirosis: Methods of Assessment. WHO Headquarters, Geneva, 25-27 October 2006.

Levett, P.N. (2001). Leptospirosis. *Clinical Microbiology Reviews*, 14(2): 296-326.

Mortimer, R.B. (2005). Leptospirosis in a caver returned from Sarawak, Malaysia. *Wilderness and Environmental Medicine*, 16(3): 129-131.

Sejvar, J., Bancroft, E., Winthrop, K., Bettinger, J., Bajani, M., Bragg, S., Shutt, K., Kaiser, R., Marano, N., Papovic, T., Tappero, J., Ashford, D., Mascola, L., Vugia, D., Perkins, B., Rosentein, N., & Eco-Challenge Investigation Team. (2003). Leptospirosis in "Eco-Challenge" Athletes, Malaysian Borneo, *Emerging Infectious Diseases,* 9: 702-707.

Smythe, L.D, Field, H.E., Barnett, L.J., Smith, C.S., Dohnt, M.F., Symonds, M.L., Moore, M.R., & Rolfe, P.F. (2002). Leptospiral antibodies in flying foxes in Australia. *Journal of Wildlife Diseases*, 38(1): 182.

Russ, A, Jali, I., Bahaman, A., Tuen, A., & Ismail, G. (2003). Seroepidemiological study of leptospirosis among the indigenous communities living in the periphery of Crocker Range Park Sabah, Malaysia. http://www.arbec.com.my/pdf/art10janmar03.pdf. Accessed March 10, 2010.

Terpstra, W.J. (2003). Human leptospirosis: guidance for diagnosis, surveillance and control, World Health Organization, International Leptospirosis Society.