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Serological prevalence of leptospiral infection in wild rats at the National Service Training Centres in Kelantan and Terengganu

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Abstract. One hundred and sixty eight rats were trapped from the National Service Training Centres (NSTC) in Kelantan and Terengganu from October 2008 to May 2009. Microscopic agglutination test (MAT) was performed to detect the presence of agglutinating antibodies to *Leptospira* among the rats caught. All the MAT positive rats were identified as *Rattus tiomanicus*. In Kelantan, 17.3 % (14/81) of the rats had leptospiral antibodies to serovars Icterohaemorrhagiae (12.3%), Canicola (2.5%), Ballum (1.2%), and Pyrogenes (1.2%). In Terengganu, 18.4% (16/87) of the rats had antibodies to serovars Icterohaemorrhagiae (15%), Canicola (1.1%), Pyrogenes (1.1%) and Hebdomadis (1.1%). This study indicated that *Leptospira* serovars were prevalent in the rat population in the study areas and could be a source of infection to humans. Therefore, control of the rat population in all NSTC is critical to prevent outbreaks of leptospirosis amongst the NSTC trainees.

INTRODUCTION

Leptospirosis is a zoonotic disease caused by pathogenic *Leptospira* which can infect both humans and animals. Rodents especially rats are the major reservoir for many leptospiral serovars. The role of rats as carriers of *Leptospira* has been investigated (Thiermann, 1981) and different species of rats have been reported to carry different pathogenic leptospiral serovars (Wangroongsarb *et al.*, 2002). In Malaysia, the role of rats as the source of human infections has been investigated by Gordon-Smith *et al.* (1961). The number of human leptospirosis cases in Malaysia has increased lately (Sejvar *et al.*, 2003). Leptospire are being maintained in the kidneys and pathogenic leptospire have been frequently isolated from wild rats (Bahaman & Ibrahim, 1988).

There are 93 National Service Training Centres with more than hundred thousand trainees' intake yearly. The trainees are

subjected to various physical activities such as obstacle courses, abseiling, canoeing among others thus predispose them to leptospiral infection from urine contaminated waters and soils (Plank & Dean, 2000). An outbreak of leptospiral infection was reported among athletes participating in the Eco-Challenge-Sabah 2000 held in Malaysian Borneo, and the infection was reported to be associated with water-related activities (Sejvar *et al.*, 2003).

Leptospirosis in humans and animals is diagnosed by culture and serological methods. Culture has many disadvantages as it takes between 3 weeks to 12 weeks to isolate the leptospire. Cultures are useful as they can ascertain which animals act as carrier for the different leptospiral serovars seen in the country. Microscopic Agglutination Test (MAT) is widely used to detect the antibodies against *Leptospira* in serum samples.

Information on the prevalence of leptospiral infection among the animal

population and identifying the predominant carrier animal species is important in control and prevention programme. This study was conducted to provide preliminary information on the prevalence of leptospiral infection among rats found in the National Service Training Centres in Kelantan and Terengganu.

MATERIALS AND METHODS

Study area and sampling procedures

Trapping of rats from the National Service Training Centres in Kelantan and Terengganu started from October to May 2009. Rats were trapped alive, anaesthetized, and blood extracted by cardiac puncture. Serum samples were kept at -20°C until time to be used.

Seroprevalence of leptospiral infection in wild rats

The microscopic agglutination test (MAT) was performed according to Faine (1982) with a panel of 14 leptospiral serovars; Australis, Autumnalis, Ballum, Bataviae, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Djasiman, Hardjobovis and Tarassovi. The leptospiral cultures were adjusted to a cell density of 1.5×10^8 cells/ml (0.5 McFarland standard) with PBS (pH 7.4) (Wangroongsarb *et al.*, 2002). Serum positive at titer 1:20 was further titrated until 1:5120. Sera were considered to be positive if the titer was $\geq 1:40$ by the MAT.

RESULTS

A total of 168 rats were caught in the NSTC in Kelantan and Terengganu. Majority of the rats caught were *Rattus tiomanicus* (94%), others were *Maxomys rajah* (3%), *Rattus argentiventer* (1.2%) and *Rattus rattus diardii* (1.8%). Positive sera for leptospiral antibodies were only seen in *R. tiomanicus* and all infected rats were adults. Seventeen of the 30 infected rats (56.7%) were males. In Kelantan, 17.3% (14/81) of the *R. tiomanicus* were positive for leptospiral

serovars by MAT; Icterohaemorrhagiae (12.3%), Canicola (2.5%), Ballum (1.2%) and Pyrogenes (1.2%). In Terengganu, 18.4% (16/87) of the rats were positive by MAT; Icterohaemorrhagiae (15%), Pyrogenes (1.1%) and Canicola (1.1%) and Hebdomadis (1.1%). MAT titers ranged from 1:40 to 1:320 in Kelantan and 1:40 to 1:1280 in Terengganu. The highest titre of 1280 was found in one sample from Terengganu was due to serovar Hebdomadis.

DISCUSSION

Cases of human leptospirosis were frequently reported to be associated with water-related activities. In Malaysia, an outbreak of human leptospirosis has been reported among athletes participating in the Eco-Challenge-Sabah 2000 held in Malaysian Borneo (Sejvar *et al.*, 2003). Patient history reported it to be related with sport activities like jungle trekking, prolonged swimming and kayaking. These activities increase the chances of contracting pathogenic leptospires from urine-contaminated waters and soils. Rodents in Malaysia have been reported as the major maintenance hosts for the various leptospiral serovars (Bahaman & Ibrahim, 1988). They shed leptospires in their urine, thus contaminating the environment.

Water-related activities exposed NSTC trainees to leptospires in the urine-contaminated environment. There has not been any report implicating wild rats as the source of pathogenic leptospires in National Service Training Centres. Leptospiral serovars have been shown to be carried by different species of rats and could have contributed to the human leptospirosis among trainees previously reported. In this study, presence of different leptospiral serovars was detected in the rats caught. This has similarly been reported in Thailand (Wangroongsarb *et al.*, 2002). From the findings, serovar Icterohaemorrhagiae was the major serovar detected in the serum samples of rats caught. This serovar is found to be associated with rodents worldwide and a major cause of clinical leptospirosis.

In this study, more male rats were detected to be positive for leptospirosis compared to female rats. Vanasco *et al.* (2003) in Argentina reported leptospiral infection were more frequently seen in male, adult wild rats. Prevalence of leptospiral antibodies tends to increase with age and in this study all positive serum samples were detected in adult rats.

To date, no study has been carried out on wild rats to implicate them as the source of leptospirosis amongst NSTC trainees. Exposure of trainees to urine-contaminated environment put them at high risk at contracting leptospirosis.

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