

SEROEPIDEMIOLOGICAL STUDY OF LEPTOSPIROSIS AMONG THE INDIGENOUS COMMUNITIES LIVING IN THE PERIPHERY OF CROCKER RANGE PARK SABAH, MALAYSIA

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INTRODUCTION

Leptospirosis has been known to affect both man and animal worldwide resulting in morbidity and mortality. Infection in domestic animals and wildlife can lead to economic loss and pose a potential spread to the communities. Man contacted the disease by direct contact with infected blood, tissues, organs or urine of infected hosts. Transmission can also occur by direct penetration of the leptospira organism through the conjunctiva or surface epithelium. Indirect contact with the environments includes soil, mud, fresh waters, vegetation, foodstuff, and working place infested with rodents (Turner 1973).

Che-Chung Tsai et al. (1975) reported the first case of leptospirosis contracted through natural infection in Asia in an individual who had exposed to wild animals while working in Vietnam. In India, Venkataraman et al. (1991) confirmed leptospirosis by urine culture and examine by dark field microscopy (DFM) in 6 of 40 urine samples obtained from patients with leptospirosis. El Jali et al. (1997) showed that ELISA was able to detect low titres of antibodies to leptospira in human and suggested a suitable technique for epidemiological studies.

In Malaysia, Fletcher (1928) reported the isolation of leptospira in black rats, *Rattus rattus* from kidneys and urine of the animal. Later Wisseman et al. (1955) successfully isolated leptospiral serovars from serogroups *Hebdomanis*, *Grippotyphosa*, *Conicola* from three species of Malaysia rodents and a serogroup of *Icterohaemorrhagiae* from *Rattus whiteheadi* from Sabah. Bahaman and Ibrahim (1986) reported that domestic animals in Malaysia that have been exposed to a large number of leptospiral serovars may act as an important maintenance hosts for leptospirosis.

This study was carried out to determine the extent of exposure to leptospirosis in the human communities living at the periphery of Crocker Range Park Sabah, an increasingly popular destination among the local and foreign tourists. In addition, small mammals were trapped in the forest habitats of Crocker Range to obtain blood serum samples for the screening of leptospiral antibodies and establish the possible zoonotic importance of wild mammals in maintenance of this disease within the communities here.

MATERIALS AND METHODS

Study sites

Several rural communities were identified in the villages situated in the periphery of Crocker

Range, Sabah Park where the communities were mainly farmers engaged in paddy cultivation, planting cash crops and breeding domestic animals. The villages identified were Sensurun, Tikoloid, Binkur, Bandukan, Ulu Senaggang and Pulung situated in the eastern region of Crocker Range, Sabah.

Samples Collection

The respondents were identified at random comprising of people between the ages of 10 to ≥ 71 years old and informed consent was given for their participation in the study. Demographic data were gathered which include name, age, sex, race, occupation and family size. Questionnaires were prepared to gather information regarding history of related illness, sanitation, housing condition and water supply. Data analysis was done by using SPSS 9.05.

Single blood samples of approximately 8cm³ were collected intravenously from the respondents and the samples were centrifuged to separate the sera which were subsequently stored at -20°C before used.

Enzyme-linked Immunoabsorbent Assay (ELISA).

The procedure used in this study was carried out as described by Bercovich et al. (1990). Linbro/Litertect EIA 96 well microtitration plates (Flow Laboratories Inc) were coated overnight at 4°C with 100 μl per well of 1/100 dilution of the antigen in 0.05M carbonate/bicarbonate buffer pH 9.6. The plate were washed six times with washing buffer containing 0.05% (v/v) Tween 20 in PBS pH 7.0. 100 μl of 1:200 diluted serum in 0.01M PBS, pH 7.2 containing 0.05% Tween was added in duplicate on each plates. Each samples was done in duplicate.

The plates were incubated at 37°C for 30 minutes and washed three times. After shaking dry, 100 μl of labeled conjugates comprising goat anti-bovine IgG peroxidase in PBS-Tween 20 was added to each wells. The plates was incubated for 30 minutes at 37°C followed by a washed for three times and 100 μl 0.4mM of ABTS Peroxidase Substrate containing 2,2'-azino-di { 3-ethylbenzthiazoline sulfonate 6 } and 2mM hydrogen peroxide in 0.05 M citrate buffer pH 4.0 were added to each well. The optical density (OD) was measured at 405 nanometers wavelength after keeping the plates in the dark for 10 minutes.

The positive and negative controls were included in each plate. Sera recorded as positive if their OD reading was two times higher than the OD reading of the negative control sera (Bercovich et al. 1990).

Small Mammal Trapping

Small mammal trappings were carried out in forest areas of CrockerRange National Park at the eastern region for a period of one week in the middle of the month of October 1999. The mammals were sacrificed for intracardiac blood samples which were screened for the presence of leptospiral antibodies.

Microscopic Agglutination Test (MAT)

The serum samples from small mammals were examined for leptospiral antibodies by MAT as describe by Cole et al. (1993).

RESULTS

A total of 299 serum samples were taken from the communities living at the periphery of the eastern region of Crocker Range Park, Sabah. A total of 77 of 299 (25.75%) serum samples were positive for antibodies to leptospiral antigen as detected by ELISA technique. The majority of the positive samples i.e. 24 of 77 (32.17%) belong to the age group of between 31-50 years old; 11 of 77 (14.29%) from the 41-50 years old age group and 10 of 77 (12.99%) from 10-20 and 61-70 years old respectively.

The majority of the communities involved in the study (43.7%) are engaged in farming; mainly of paddy cultivators while others work as laborers (2.7%). The levels of education of the communities are mainly primary (38%), secondary (33.3%), tertiary (2.7%) and adult education (1.3%). As high as 21.3% of them had not attended any formal schooling.

Only 54.3% of the population surveyed received adequate chlorinated water supply and 40.0% did not have properly treated water supply or obtained their daily requirement of water from the nearby rivers. Most of the households have adequate toilet facilities in the form of pit latrines (57.3%), pour latrines (35%) and flush latrines (5.7%).

The majority of the households surveyed have an income of less than RM300 per month and the majority of them have large family size ranging from 5-9 members (74.7%).

Sixteen blood samples were collected from a variety of small mammals trapped in the forest areas of the Crocker Range on the eastern region of the Park. The number of mammals caught include four individuals of *Tupaia montana*, two individuals of *Myxomys surifer* and *Leopoldamys sarbanus* and a single individual each of *Tupaia minor*, *Brook squirrel*, *Cynopterus horsfieldi*, *M. whiteheadi*, *T. montana*, *C brachyotis*, *Megaerops ecaudatus* and *Niviventer cremoriventer*. Our tests however revealed that all species were negative for antibodies to serovar *Leptospira hadjo*, *L.pomona* and *L. australis* using Mat Agglutination Test (MAT).

DISCUSSION

This study showed a significant percentage (25.75%) of the people living within the periphery of the Crocker Range Park has been exposed to leptospiral antigens. The high rate of exposure to leptospirosis could have been related to the daily activities in the livelihood of the indigenous communities that bring them in close contact with the natural forested and riverine environment inhabited by mammalian hosts of the disease. This is particularly plausible as a potential occupational hazard for people engaged in agricultural activities in paddy cultivation, livestock keepers and those working or gathering food and medicines from the forests. The people in these villages rely on the rivers flowing from the Crocker Range for their water supply which serves also as their drinking water, domestic needs, washing, and agricultural activities. They may be exposed to leptospirosis through the consumption of waters drawn from contaminated rivers or bathing in them. The risk of contact occurs mostly after heavy downpours upstream where contaminated urine of wild rodents are washed into the rivers from the forest floors.

Another risk factor includes exposure to leptospira from rodents that lives within the human communities themselves especially in overcrowded and unsanitary conditions. Wisseman (1955) reported the isolation of a serovar of *Leptospira icterohaemorrhagiae* in Sabah. Our own study to detect the presence of antibodies to serovar *L. hadjo*, *L. pomona* and *L. australis* in small mammals trapped in the forest of Crocker Range proved negative. However our result may be flawed by the limited number of samples obtained during a short period of field work. A more intensive effort is required in the screening of serovar leptospiral antibodies in mammalian hosts in our forest environment to give an indication of the potential zoonotic importance of this disease among the indigenous communities of rural Sabah. The serovar screening for leptospirosis for example must also include agricultural and domestic animals found living in association with the rural communities.

Bahaman (1988) reviewed that rodents, domestic animals and dogs play a major role as the maintenance hosts of the various serovars of leptospira. Cattle and buffalo for instance are natural hosts for serovar *L. hadjo* where the leptospira are known to survive in the host for many years; passing the leptospira through the urine into the environment. Similarly, *L. icterohaemorrhagiae* use rodents as its natural host and *L. pomona* are found in pigs. Although, our screening of small mammals from the forests of Crocker Range Park failed to reveal the presence of antibodies for three serovar of *Leptospira hadjo*, *L. pomanis* and *L. arustralis*. the study did not rule out the possibility of detecting antibodies against other medically important *Leptospira* serovars if provided with more animal samples from the study areas.

General precaution has to be observed by visitors to these forested areas when using the river water or bathing in rivers in the Park; particularly swimming in the contaminated rivers after a downpour. Personal hygiene is essential in recreational activities to prevent contact with the leptospiral organisms found within the seemingly pristine forest environment and clean rivers.

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