Faculty of Medicine and Health

SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF LEPTOSPIROSIS IN HUMAN AND SMALL MAMMALS IN THE REJANG BASIN

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Declaration

I hereby declare that this thesis entitled “Seroprevalences and Associated Risk Factors of Leptospirosis in Human and Small Mammals in the Rejang Basin” is the result of my own research work and effort. No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification at any other university or institution of higher learning. In instances, where other sources of information have been used, they have been appropriately acknowledged.

Signature:

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Date:
Acknowledgement

In the name of Allah, the Merciful, the Compassionate.

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Abstract

Leptospirosis is an important zoonotic disease in the world. The wet and warm climate in the Southeast Asia region provides a favourable condition for leptospires to survive in the environment and pathogenic leptospires have frequently been isolated from Malaysian waters and soils. A study was conducted in the Rejang basin of Sarawak from June 2009 to June 2012 to determine the seroprevalence of leptospirosis in the population and small mammals. The Rejang basin was chosen because leptospirosis cases had been previously found there. For human samples, all 508 sera were tested with Panbio® ELISA for leptospira IgM and the positive cases will be further examined by microscopic agglutination test (MAT) using 20 serovars obtained from the Institute of Medical Research (IMR), Kuala Lumpur. The seroprevalence of leptospirosis in human in the study area is 37.4% (190/508). The presumptive infecting serovars found were djasiman (22.1%), shermani (13.2%) and pomona (7.9%).

For small mammals, 241 were trapped during the study and species that were trapped were Sundamys muelleri with 100 individuals (41.5%) (100/241), followed by Callosciurus notatus with 46 individuals (19.1%) (46/241), and Rattus exulans with 45 individuals (18.7%) (45/241). All blood extracted from small mammal samples were tested by MAT and ninety-eight (98) of sera samples tested (40.7%) (98/241) were positive for the antibody against Leptospira sp. with predominant presumptive serovars comprising of autumnalis (26.3%), tarassovi (23.2%) and bataviae (15.2%).

The median age of seropositive cases was 42 years, many of whom were reliant on collected rain water and nearby river (74.7%) as their main water supply. More than half (56.3%) of the positive cases reported of having fever within the last 2-4 weeks before samples collection. Almost sixty percent (59.5%) of the seropositive respondent are females, whom are mostly housewives or farmers. Serovars australis, autumnalis, bataviae, canicola,
celledoni, copenhageni, hebdomadis, tarassovi, pyrogenes, icterohaemorrhagiae, ballum, shermani and panama can be correlated to both seropositive human and small mammals. Based on the result, Rejang Basin had the highest leptospirosis prevalence among human population and differences serovars compared to previous studies done in Malaysia.

Logistic regression was used to identify factors associated with the leptospirosis infection. Factors that have significant association with the presence of leptospiral antibodies based on univariate analysis were: Primary education (OR= 0.654; 95% CI= 0.414-1.033), Secondary education (OR= 0.363; 95% CI= 0.219-0.602), presence of symptoms (OR=1.582; 95% CI=1.041-2.404), age groups of 11-20 years (OR= 0.291; 95% CI= 0.095-0.889), 21-30 years (OR= 0.209; 95% CI= 0.072-0.608), 41-50 years (OR=0.323; 95% CI=0.115-0.911), 51-60 years (OR= 0.396; 95% CI= 0.137-1.141), Sarikei division (OR=0.360; CI= 0.169-0.766) and contact with animal (OR= 0.511; 95% CI= 0.279-0.937). Based on multivariate analysis, the respondents’ educational level, age group and history of contact with animals remain to be significant. The respondents whom were defined as having the probable infection at time of survey were 5.3 times more likely to be symptomatic. The result also suggested that leptospirosis cases in Rejang Basin were most likely due to exposure to leptospires in the environment, possibly originating from the animal carriers. Due to the high prevalence of leptospirosis in the study area, appropriate public health and control strategies targeting this population should be implemented.
Abstrak

Leptospirosis adalah penyakit zoonotik yang penting di dunia. Iklim yang lembab dan panas di rantau Asia Tenggara menyediakan keadaan yang sempurna untuk *Leptospira sp.* untuk hidup dan leptospira patogenik telah kerap diasingkan dari air dan tanah di Malaysia. Satu kajian telah dijalankan di lembah Rejang Sarawak dari Jun 2009 hingga Jun 2012 untuk menentukan seroprevalens leptospirosis di kalangan penduduk dan mamalia kecil. Lembah Rejang telah dipilih kerana terdapat kes leptospirosis telah dilaporkan di sana. Untuk sampel penduduk, kesemua 508 serum telah diuji untuk IgM leptospira menggunakan Panbio® ELISA dan sampel yang positif akan menjalani ujian *microagglutination test* (MAT) menggunakan 20 serovar yang diperolehi dari Institut Penyelidikan Perubatan (IMR), Kuala Lumpur. Seroprevalens untuk leptospirosis bagi penduduk di kawasan kajian adalah 37.4% (190/508). Serovar yang paling kerap menjangkiti penduduk di kawasan kajian adalah djasiman (22.1%), shermani (13.2%) dan pomona (7.9%).

Sebanyak 241 ekor mamalia kecil telah diperangkap dan species yang telah ditangkap adalah *Sundamys muelleri* sebanyak 100 individu (41.5%) (100/241), diikuti oleh *Callosciurus notatus* sebanyak 46 ekor (19.0%) (46/241), dan *Rattus exulans* sebanyak 45 ekor (18.6%) (45/241). Kesemua serum yang diperolehi dari mamalia kecil diuji dengan MAT dan sembilan puluh lapan (98) daripada serum yang diuji (40.7%) (98/241) adalah positif untuk antibodi dari *Leptospira sp.*, dan serovar yang kerap dikesan pada haiwan-haiwan di kawasan kajian ialah autumnalis (26.3%), tarassovi (23.2%) and bataviae (15.2%).

Median umur untuk kes seropositif adalah 42 tahun, dan kebanyakan mereka bergantung kepada air hujan dan sungai berdekatatan (74.7%) untuk bekalan air utama mereka. Lebih separuh (56.3%) dari kes positif dilaporkan mengalami demam dalam tempoh 2-4 minggu sebelum darah mereka diambil. Hampir enam puluh peratus (59.5%) responden seropositive adalah wanita, yang kebanyakannya suri rumah atau petani. Serovar australis,
autumnalis, bataviae, canicola, celledoni, copenhageni, hebdomadis, tarassovi, pyrogenes, icterochaemorraghiae, ballum, shermani and panama dapat dikaitkan diantara leptospirosis di kalangan manusia dan mammalia kecil. Berdasarkan daripada keputusan, Lembah Rejang mmpunyai prevelansi leptospirosis yang tertinggi bagi penduduk dan mempunyai serovar yg berbeza jika dibandingkan dengan kajian-kajian yang telah dijalankan di Malaysia.

Ujian regresi logistik telah digunakan untuk mengenal pasti faktor-faktor yang dapat dikaitkan dengan jangkitan leptospirosis. Faktor-faktor yang mempunyai hubungan signifikasi dengan kehadiran antibodi leptospirosis berdasarkan analisis univariat adalah: tahap pendidikan rendah (OR= 0.654; 95% CI= 0.414-1.033), pendidikan menengah (OR= 0.363; 95% CI= 0.219-0.602), kehadiran simptom (OR=1.582; 95% CI=1.041-2.404), kumpulan umur 11-20 tahun (OR= 0.291; 95% CI= 0.095-0.889), 21-30 tahun (OR= 0.209; 95% CI= 0.072-0.608), 41-50 tahun (OR=0.323; 95% CI=0.115-0.911), 51-60 tahun (OR= 0.396; 95% CI= 0.137-1.141), Bahagian Sarikei (OR=0.360; CI= 0.169-0.766) dan mempunyai kontak dengan haiwan (OR= 0.511; 95% CI= 0.279-0.937). Berdasarkan analisis multivariat, tahap pendidikan, kumpulan umur dan sejarah kontak dengan haiwan masih kekal signifikan. Responden yang disyaki mengalami jangkitan semasa kajian dijalankan adalah 5.3 kali untuk menunjukkan simptom. Keputusan yang diperolehi juga mencadangkan bahawa kes leptospirosis di Lembah Rejang berpunca dari persekitaran. Disebabkan tahap prevelansi yang tinggi di kawasan kajian, tindakan dan kawalan strategi oleh pihak kesihatan awam yang mensasarkan penduduk ini perlu dilaksanakan.
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Table 5.50. The predicted probability of being positive for leptospiral antibody based on several factors
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>Celcius</td>
</tr>
<tr>
<td>µl</td>
<td>Microlitre</td>
</tr>
<tr>
<td>µm</td>
<td>Micrometre</td>
</tr>
<tr>
<td>am</td>
<td>Ante Meridiem</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CAL</td>
<td>Calibrator</td>
</tr>
<tr>
<td>CFT</td>
<td>Complement fixation test</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DST</td>
<td>IgM dot-ELISA dipstick test</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EMJH</td>
<td>Ellinghausen-McCullough-Johnson-Harris</td>
</tr>
<tr>
<td>FAT</td>
<td>Fluorescent antibody test</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IHA</td>
<td>Indirect Hemagglutination Assay</td>
</tr>
<tr>
<td>IMR</td>
<td>Institute of Medical Research</td>
</tr>
<tr>
<td>JKNS</td>
<td>Jabatan Kesihatan Negeri Sarawak</td>
</tr>
<tr>
<td>JPN</td>
<td>Japan</td>
</tr>
<tr>
<td>km</td>
<td>kilometre</td>
</tr>
<tr>
<td>LAMP-1</td>
<td>Lysosomal-associated membrane protein 1</td>
</tr>
<tr>
<td>LDS</td>
<td>IgM dipstick assay</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>m</td>
<td>metre</td>
</tr>
<tr>
<td>MAT</td>
<td>microscopic agglutination test</td>
</tr>
<tr>
<td>MCAT</td>
<td>One-point Microcapsule Agglutination Test</td>
</tr>
<tr>
<td>mg</td>
<td>Miligram</td>
</tr>
<tr>
<td>MOH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
</tr>
<tr>
<td>NST</td>
<td>New Strait Times</td>
</tr>
<tr>
<td>O.D</td>
<td>Optical density</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PFGE</td>
<td>Pulsed-field gel electrophoresis</td>
</tr>
<tr>
<td>pm</td>
<td>Post Meridiem</td>
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<tr>
<td>REA</td>
<td>Restriction Endonuclease Analysis</td>
</tr>
<tr>
<td>RSAT</td>
<td>Rumah Sakit Angkatan Tentera</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>sq.km</td>
<td>Square kilometre</td>
</tr>
<tr>
<td>TMB</td>
<td>3,3',5,5'-tetramethylbenzidine chromogen</td>
</tr>
<tr>
<td>UN</td>
<td>United Nation</td>
</tr>
<tr>
<td>UNIMAS</td>
<td>Universiti Malaysia Sarawak</td>
</tr>
<tr>
<td>USA</td>
<td>United State of America</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WWF</td>
<td>World Wildlife Fund</td>
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<tr>
<td>UK</td>
<td>United Kingdom</td>
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</table>
1 Problem statement

Leptospirosis is a re-emerging zoonotic disease that may affect both human and animals. Leptospirosis is caused by *Leptospira interrogans*, a gram-negative, helical, thin, actively motile spirochaete organism that affects many animal species and human (World Health Organization, 2003). Leptospira are classified according to serovar status and more than 230 pathogenic serovars have been identified (Bharti *et al.*, 2003; Smythe *et al.*, 2002). The wet and warm climate in the Southeast Asia region provides a favourable condition for leptospires to survive in the environment and pathogenic leptospires have been isolated from Malaysian waters and soils (Alexander *et al.*, 1975; Fletcher, 1928; Khairani and Bahaman, 1997).

Leptospirosis is common in rodents and other wild and domestic mammals, where infection occurs in approximately 160 mammalian species (Alexander, 1991). Each serovar has its preferred animal host or hosts and the geographic region, but each animal species may be host to one or more serovars. There are increased numbers of leptospirosis cases throughout Malaysia. This may be caused by either a true increase number of cases of leptospirosis in the region or possibly due to increased awareness regarding the disease.

In Sarawak (2004-2009), the number of leptospirosis cases reported was lower than seven other states in Malaysia such as Perak, Selangor, Pahang, Kelantan, Terengganu, Kedah and Negeri Sembilan (MOH, 2011). Although there are many factors that may suggest that leptospirosis cases in Sarawak is much higher, lack of awareness and diagnostic ability in the state may obscure the actual number of leptospirosis cases. Sarawak also lacks the resources for leptospira full diagnostic work up. Sarawak is the 3rd lowest state with ratio of doctor to population with 1:1491 compared to Malaysia average with 1:859. Although Malaysia had exceed the 1,600 health care facilities to meet the metric of one health facility in every 5 km radius, the distribution is uneven especially in Sarawak where fairly large
number of rural people do not have a health facility within a 5 km or 10 km radius (United Nation, 2011). In recent years (2010 onwards) in Malaysia in general, and in Sarawak specifically, the number of mortality associated with leptospirosis had shown a rising trend (Thayaparan et. al., 2013a).

Since the number of cases reported mainly came from hospitals based data, it is difficult to gauge the magnitude of the problem that leptospirosis posed. Furthermore, most of the studies regarding prevalence of leptospirosis were conducted in other countries, and the most recent data regarding prevalence of the disease in Peninsular Malaysia was done about a decade ago. For Sarawak, no comprehensive study regarding the prevalence of leptospirosis has been attempted thus far. It is thought that the population characteristics of Sarawak may predispose them to increased risk of exposure to the pathogenic Leptospira sp.. Quite a number of the populace in rural parts of Sarawak are engaged in agricultural activities, use the river as one of the main transportation mode and water source, and source out jungle produce as part of their economic activities. The above factors with the availability of the animal hosts of the microorganism and suitable weather conditions may contribute to a greater likelihood of exposure to the pathogenic leptospires either by direct or indirect contact. It is imperative that a prevalence study be conducted in Sarawak to estimate the burden of the disease. Rejang basin was chosen as a suitable area for this study as its geographical variation such as mountainous terrain to sea coast, and economical activities of the population fitted the above criteria. In addition, the Rejang basin encompassed a vast area and it represented about a third of Sarawak land area, agricultural-based, and is seen as a suitable area where the above factors interact.

It is anticipated that the findings from this study can be utilised to understand the factors associated with leptospirosis, potential animal reservoir and the infecting serovars of the pathogenic Leptospira sp. in Sarawak. These informations will increase our understanding
regarding leptospirosis burden in Sarawak and possibly heightened public awareness regarding the disease. The findings are expected to be of assistance and be utilised towards planning an effective eventual prevention and control of leptospirosis in Sarawak, and possibly Malaysia in general.

1.1 Aim of study

This study aimed to study the extent of exposure to leptospirosis among the population of Sarawak, and its associated risk factors by focusing on the Rejang basin. It is a vast area, which encompasses an area of 51,546 sq.km and inhabited by 631,416 populations (Malaysia 2010 census) (Department of Statistics Malaysia, 2011). The geography of Rejang basin and its population characteristics, as described above render it as a suitable study area for leptospirosis.

1.2 Study hypothesis

- The prevalence of leptospirosis in the Rejang basin, Sarawak does not differ from previous published studies in Malaysia.

1.3 General Objectives

To determine the seroprevalence and distribution of leptospirosis in the Rejang basin, Sarawak
1.4 Specific Objectives

- To determine the seroprevalence and distribution of leptospirosis among the population in the Rejang basin, Sarawak.
- To identify the common infecting serovars of leptospira in the Rejang basin population
- To determine the risk factors associated with leptospira seropositivity among the Rejang basin population.
- To determine the seroprevalence and distribution of leptospirosis in trapped small mammals in the Rejang basin, Sarawak.
- To correlate the common infecting serovar of leptospira found in seropositive individuals and animals trapped in the Rejang basin.
2 Literature review

2.1 Leptospirosis

Leptospirosis is an infectious disease caused by several immunologically distinct serovars of the genus *Leptospira*, a gram-negative, helical, thin, spiral-shaped bacteria, with open, hooked ends; they are motile, aerobic, and culturable, and they measure some 6 to 20 microns long by 0.1 microns in diameter, actively motile spirochaete organism affecting many animal species and human (World Health Organization, 2003). They can be seen under a dark-field microscope and pass through filters that block other bacteria. *Leptospira* are classified according to serovar status with more than 230 pathogenic serovars have been identified (Bharti et. al., 2003; Smythe et. al., 2002). Some of the serovar that had been identified are australis, autumnalis, bataviae, canicola, celledoni, grippotyphosa, hardjobovis, hebdomadis, icterohaemorrhagiae, javanica, pyrogenes, djasiman, patoc, ballum, pomona, copenhageni, shermani, panama and tarassovi.

The disease can be transmitted by wild or domestic animals, especially rodents and small marsupials, cattle, pigs and dogs. Almost every mammal including aquatic mammals and marsupial has been known to be a carrier of leptospires (Faine et. al., 1999). Human transmission of leptospirosis disease usually occurs indirectly through damaged skin or mucus membranes exposed to contaminated or infected urine, foetal fluids as well as water, moist soil or vegetation contaminated with urine from infected animals (Mgode et. al., 2006; Radostitis et. al., 2000).

The term *Leptospira* was introduced by Noguchi (Champagne et. al., 1991) in recognition of its difference from other spirochaetes. On morphological grounds the Family Leptospiraceae, among the Order Spirochaetales, was first published by Pillot and Ryter (1965). There are no anatomical features that generally can be used reliably to differentiate within the major groups of leptospires.
2.2 *Leptospira sp.* Classification

### Leptospira

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Monera</th>
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<tbody>
<tr>
<td>Phylum</td>
<td>Spirochaetes</td>
</tr>
<tr>
<td>Class</td>
<td>Spirochaetes</td>
</tr>
<tr>
<td>Order</td>
<td>Spirochaetales</td>
</tr>
<tr>
<td>Family</td>
<td>Leptospiraceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Leptospira</td>
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Leptospires are thin, helically coiled, motile spirochetes usually 6–20 μm in length but occasional cultures may contain much longer cells. The helical amplitude is approximately 0.1 to 0.15 μm, and the wavelength is approximately 0.5 μm (Faine *et al.*, 1999). The end-hooked appearance gave its distinctive question-mark shape as shown in Figure 2.1.

![Figure 2.1. Scanning electron micrograph of *L. interrogans* serovar icterohaemorrhagiae strain RGA bound to a 0.2-mm membrane filter. Taken from Weyant *et al.* (1999).](image-url)
The surface structures of leptospires resemble both Gram-positive and Gram-negative bacteria. The double-membrane and the presence of lipopolysaccharide (LPS) are characteristic of Gram-negative bacteria in common with other spirochetes where the cytoplasmic membrane and peptidoglycan cell wall are closely associated and are overlain by an outer membrane (Haake et al., 2000). Leptospiral LPS has a composition similar to that of other gram-negative bacteria (Vinh et al., 1986) but has lower endotoxic activity (Shimizu et al., 1987). Close association of the cytoplasmic membrane with murein cell wall is reminiscent of Gram-positive envelope architecture (Haake et al., 2000; Ko et al., 2009; Levett, 2001; Vijayachari et al., 2008).

Motility in leptospires is provided by two periplasmic flagella or endoflagella which are located at each end of the bacterium. The structure of the flagellar proteins is complex (Trueba et al., 1992). Leptospires exhibit two distinct forms of movement, translational and nontranslational (Berg et al., 1978). Freshly isolated pathogenic leptospires are usually more tightly coiled and shorter than laboratory strains after several culture passages (Ellis et al., 1983). The direction of the coiling is right-handed (Carleton et al., 1979; Yoshii, 1978).

Before 1989, the genus Leptospira was used to be divided into two species, L. interrogans which comprised all pathogenic strains and L. biflexa which contained the saprophytic strains isolated from the environment (Faine and Stallman, 1982; Johnson and Faine, 1984). The large numbers of serovars within each species were further recognised and differentiated using agglutinating antibodies (Levett, 2001).

Nowadays leptospires are classified into a number of species which were defined by their degree of genetic relatedness that was determined by DNA reassociation (Brenner et al., 1999; Yasuda et al., 1987). There are currently 14 named species, including pathogens (e.g., L. interrogans), nonpathogenic saprophytes (e.g., L. biflexa), and species of indeterminate pathogenicity (e.g., L. inadai) (Faine et al., 1999).