

SEROPREVALENCE OF LEPTOSPIROSIS AMONG TOWN SERVICE WORKERS IN KELANTAN, MALAYSIA

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Abstract. Leptospirosis is presumed to be the most widespread re-emerging zoonotic disease of global distribution affecting humans in tropical, subtropical and temperate zones. Humans can be exposed to leptospirosis as a result of variety of occupations, such as town service worker, paddy planter, army and health care worker due to the presence of environmental determinants for human leptospirosis. This study determined seroprevalence of leptospirosis among 321 town service workers in Kelantan, a northeastern state of Malaysia, involving a cross sectional study in four districts, namely, Kota Bharu, Gua Musang, Kuala Krai, and Tanah Merah in Kelantan. Sociodemographic data were collected and sera samples screened for the presence of anti-leptospiral antibodies using IgM enzyme-linked immunosorbent assay (IgM ELISA) and confirmed by microscopic agglutination test (MAT) using a panel of 20 live leptospire obtained from Institute of Medical Research (IMR) Kuala Lumpur, Malaysia. Serovars used were australis, autumnalis, bataviae, canicola, celledoni, copenhageni, djasiman, grippotyposa, hardjobovis, hardjoprajitno, icterohaemorrhagiae, javanica, lai, melaka, pomona, pyrogenes, patoc, sarawak, tarassov, and terengganu. A MAT titer of ≥ 100 was used as cut-off for positive samples. All participants were of Malay race, with mean age (\pm SD) of 40 (\pm 10) years and mean duration of employment of 12 (\pm 10) years. Ninety-eight samples were positive or indeterminate by IgM ELISA, and MAT revealed an overall seroprevalence of leptospirosis of 25.5% (95% CI : 20.6-30.5). Garbage collectors had the highest seroprevalence (35%; 95% CI : 8.1-14.9), followed by town cleaners (30%; 95% CI : 43.6-54.8), lorry drivers/mechanics (20%; 95% CI : 17.8-27.4), and landscapers (14%; 95% CI : 13.1-21.8). The most common serovar was against a strain of *Leptospira* spp isolated from Sarawak (60%) and none positive for serovars bataviae, djasiman, hardjoprajitno, melaka, pyrogenes, pomona and terengganu. The present study highlights the high risk of leptospiral infection among town service workers in Kelantan, Malaysia.

Keywords: high risk occupation, leptospirosis, seroprevalence, town service worker

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INTRODUCTION

Leptospirosis is considered to be the most widespread re-emerging zoonotic disease of global distribution affecting humans in tropical, subtropical and temper-

ate zones. The most significant foci of the disease are The Caribbean, Latin America, the Indian subcontinent, Southeast Asia, Oceania, and Eastern Europe (Pappas *et al*, 2008). Seychelles was found to have the highest prevalence of leptospirosis in the world with an annual rate of 432.1 per million population while USA reported the lowest annual rate of 0.1 per million population (Pappas *et al*, 2008). The exact number of human cases worldwide is not known due to a lack of comprehensive surveillance (Hartskeerl *et al*, 2011).

Incubation period of leptospirosis infection is usually 5-14 days, with a range of 2-30 days (WHO, 2012). In humans, it can cause a wide range of symptoms, ranging from mild (influenza-like illness symptoms) to severe (Weils' syndrome), while some infected persons may have no symptoms at all. If the infection is not treated, progression of the disease will lead to complications, such as renal failure, meningitis, liver damage, respiratory distress and widespread hemorrhage (Dircio Montes Sergio *et al*, 2012).

Humans may be exposed to leptospirosis through occupational, recreational or environmental factors (Levett, 2001). Occupation such as town service worker, paddy planter, army and health care worker impose higher risk of leptospiral infection due to presence of environmental determinants for human leptospirosis (Hadad *et al*, 2006; Tiwari, 2008; Sulong *et al*, 2012). Town service workers are more likely to be exposed to the leptospiral infection as a result from their occupational activities in every step of solid waste management process. Based on data from an in-depth, long term and country-wide studies in Denmark, disease and injury risks for solid waste management workers are six times greater than those of control population (Cointreau, 2006).

Thus, this study was conducted to determine the seroprevalence of leptospirosis among town service workers in Kelantan, Malaysia.

MATERIALS AND METHODS

Study population

A cross sectional study was carried out in four districts (Kota Bharu, Gua Musang, Kuala Krai, Tanah Merah) in Kelantan, a northeastern state of Malaysia, involving 321 town service workers: garbage collector, town cleaner, landscaper and lorry driver/mechanic. Garbage collector collects garbage from residential, commercial and industrial areas and dumps garbage from containers onto lorry (truck) for disposal at landfills; town cleaner duties are sweeping, collecting and removing of litter, detritus and leaves from public spaces; landscaper performs a range of duties, such as transporting and planting new vegetation, mulching, fertilizing, watering, as well as cutting and trimming grass; and lorry driver, in addition to routine work, sometimes assists garbage collectors to perform their duties, while lorry mechanic repairs and undertakes maintenance of the lorries used for garbage collection. Sample size, calculated based on 24.7% seropositivity for leptospirosis among town service workers (Sulong *et al*, 2012) at 95% confidence interval (CI) and a drop-out of 20%, was estimated to be 346. Participants were interviewed for socio-demographic, job category and duration of employment. Data were analyzed using SPSS Version 20.0 (IBM, Armonk, NY).

Prior written consent was obtained from participants and ethical clearance was approved by Research and Ethic Committee (Human), School of Medical Sciences, Health Campus, Universiti Sains

Table 1
Sociodemographic characteristics of
town service workers, Kelantan,
Malaysia.

Characteristic	Number (%) (N = 321)
Age (year), mean (SD)	41 (10)
Gender	
Male	309 (96)
Female	12 (4)
Marital status	
Married	268 (83)
Single	44 (14)
Widower	9 (3)
Number of children, mean (SD)	4 (2)
Monthly income (RM), mean (SD)	1,198 (419)
Education level	
No formal education	4 (1)
Primary school	64 (20)
Lower secondary school	88 (28)
Upper secondary school	151 (47)
Form 6 / Diploma	14 (4)
Town council	
Kota Bharu Municipal Council	183 (57)
Gua Musang District Council	48 (15)
Kuala Krai District Council	49 (15)
Tanah Merah District Council	41 (13)
Job category	
Town cleaner	157 (49)
Lorry driver/mechanic	71 (22)
Landscaper	56 (17)
Garbage collector	37 (12)

Malaysia [Reference no: USMKK/PPP/JEPeM 261.3(7)].

Sample analysis

Five ml of venous blood were obtained from each participant and stored at 4-8°C until used. Serum samples were screened for the presence of anti-leptospiral antibodies using IgM enzyme-linked immunosorbent assay (IgM ELISA) and confirmed by microscopic agglutination

test (MAT) following standard protocols (WHO, 2007). MAT was performed with a panel of 20 live leptospires obtained from Institute of Medical Research (IMR), Kuala Lumpur, Malaysia. Serovars used were australis, autumnalis, bataviae, canicola, celledoni, copenhageni, djasiman, gripotyposa, hardjobovis, hardjoprajitno, icterohaemorrhagiae, javanica, lai, melaka, pomona, pyrogenes, patoc, sarawak, tarassov, and terengganu. MAT titer of ≥ 100 was taken as cut-off for a positive sample. Seroprevalence of leptospirosis was described with 95% CI.

RESULTS

Three hundred and twenty-one town service workers were enrolled in the study. All participants were of Malay race with a mean \pm SD age of 41 ± 10 years, with mean duration of employment of 12 ± 10 years. Majority of them were from Kota Bharu municipal council and work as town cleaner. Their education status mainly at the level of upper and lower secondary school (Table 1).

Ninety-eight serum samples from 321 subjects (30.5%) were positive or indeterminate in the IgM-ELISA. Subsequent MAT revealed an overall seroprevalence of leptospirosis of 25.5% (82/321) (95% CI: 20.6-30.5). Garbage collectors had the highest seroprevalence (35.1%; 95% CI: 8.1-14.9), followed by town cleaners (30%; 95% CI: 43.6-54.8), lorry drivers/mechanics (20%; 95% CI: 17.8-27.4), and landscapers (14%; 95% CI: 13.1-21.8) (Table 2).

The most common serovar among the 82 seropositive subjects as determined by positive MAT with titer ≥ 100 was sarawak (60%) (Table 3). None of the samples were positive for serovars bataviae, djasiman, hardjoprajitno, melaka, pyrogenes, pomona and terengganu.

Table 2
Seroprevalence of leptospirosis using MAT among town service workers according to job categories.

Job category	Number	MAT ≥ 100	
		Number (%)	95% CI
Town cleaner	157	47 (30)	43.6-54.8
Lorry driver/mechanic	71	14 (20)	17.8-27.4
Landscaper	56	8 (14)	13.1-21.8
Garbage collector	37	13 (35)	8.1-14.9
Total	321	82 (25.5)	20.6-30.5

Table 3
Serovar determined by positive MAT (titer ≥ 100) among town service workers (N = 82).

Serovar tested	Number positive (%) ^a
sarawak	49 (60)
grippotyphosa	20 (24)
patoc	19 (23)
copenhageni	9 (11)
lai	8 (10)
celledoni	4 (5)
javanica	4 (5)
australis	3 (4)
canicola	3 (4)
hardjobovis	2 (2)
icterohaemorrhagiae	2 (2)
autumnalis	1 (1)
tarrasovi	1 (1)

^aTotal >100 as workers can be positive for more than one serovar.

DISCUSSION

MAT is the ‘gold standard’ test for the diagnosis of leptospirosis. It is also used for seroprevalence survey and is generally performed by reference laboratories (Chirathaworn *et al*, 2014). In a

seroprevalence survey, the cut-off value of MAT depends on endemicity of the study area and varies from one reference laboratory to another (Plank and Dean, 2000; WHO, 2007). Currently, there is no consensus with regards to standard cut-off titer for leptospirosis seroprevalence studies in Malaysia; however, the cut-off titer value of ≥ 100 is generally accepted (Plank and Dean, 2000) and is frequently used in other seroprevalence studies all around the world including Malaysia (Gonçalves *et al*, 2006; Swapna *et al*, 2006; Majd *et al*, 2012; Sulong *et al*, 2012).

This seroprevalence study for leptospirosis among town service workers in Kelantan, Malaysia revealed the highest seroprevalence in the occupational groups studied compared to previous similar surveys which reported seroprevalence of 16.2% to 25.5% (Tan, 1973; Sulong *et al*, 2012). MAT seroprevalence in our study was comparable to that of Sulong *et al* (2012). The highest prevalence was noted among garbage collectors. In comparison to previous study (Sulong *et al*, 2012), the present study reported higher seroprevalence in all job categories except for landscapers. The higher seroprevalence among garbage collectors was probably related to their daily occupational activi-

ties that prolonged exposure and contact with garbage, soil, water or environment that might be contaminated with leptospire from urine of infected animal hosts.

Seroprevalence for leptospirosis among town service workers in this study was higher than among army personnel in Malaysia, 16.2% by Yaacob *et al* (2013, unpublished data) and 17.2% in an earlier study by Tan and Lopes (1972). Based on sensitized-erythrocyte-lysis (SEL) test, 24.2% of blood samples from healthy paddy planters in Kelantan showed leptospiral antibodies in the wet season but only 7.2% during the dry season (Tan, 1970). Oil palm estate workers recorded the highest seroprevalence of 32.6%, followed by hospital staff (25.5%) and rubber estate workers (23.2%) (Tan, 1973). Leptospirosis seroprevalence ranged from 11.6% to 16.4% in veterinary staff, policemen, shop owners, anti-malarial laborers, farmers and tin miners, whereas in occupational low risk group (school teachers, office workers and housewives), prevalence ranged from 1.5% to 9.4% (Tan, 1979).

It was difficult to relate the findings of the predominant serovars among town service workers in this study to local animal reservoirs and environment as there is limited study on human, animal and environmental samples (water and wet soils) leptospirosis in Malaysia and in Kelantan in particular (Thayaparan *et al*, 2013). *L. interrogans* serovar sarawak (also known as lepto 175), the most predominant serovar found in our study, is a poorly known strain endemic to Malaysia and its pathogenic potential is still unknown. Ongoing research to obtain more information on serovar Sarawak especially in human leptospirosis infection is currently being undertaken. In wildlife in Sarawak state, Malaysia positive titer for

serovar Sarawak is found in monkey, rat, bat, squirrel and mongoose (Thayaparan *et al*, 2013). Rats are well-known carriers of leptospirosis, which they spread among other animals and humans; however, their carrier status of the other infected animals are not known.

Previously, in a study among town service workers of Kota Bharu Municipal Council in 2008 the common serovars identified are patoc (39.7%), followed by bataviae (12.3%) and javanica (11.0%), and no sample was positive for serovar grippotyphosa, but serovar sarawak was not tested (Sulong *et al*, 2012). Serovar patoc was used as a saprophytic strain that cross-reacts with human antibodies generated by a number of pathogenic serovars, including those of rare, unknown or new strains in the area concerned (WHO, 2003). This may suggest the circulation of other pathogenic serogroups that were not included in the MAT antigens of 20 serovars used in the present study. These findings emphasize the need to validate or revise the MAT criteria for the specific epidemiological situation in which serosurveys are or will be performed.

A study involving army personnel, one of the occupation risk group, in four base camps (Pengkalan Chepa, Desa Pahlawan, Tanah Merah and Jeli) of Kelantan reported the common serovars detected are pathogenic serovar terengganu (38.3%) and non-pathogenic serovar patoc (35.2%), but MAT for serovar tarrasovi, djasiman and grippotyphosa were not performed (Yaacob *et al*, 2013, unpublished data). Serovar terengganu, together with serovars melaka, sarawak and bejo, are local strains in Malaysia, but very limited published studies are available (Slack *et al*, 2009; Thayaparan *et al*, 2013). A hospital-based study involving 999 febrile cases from 10 hospitals

in Kelantan reported the most common serogroup is Sejroe, which accounts about 82% of the leptospirosis cases (Rafizah *et al*, 2013). Seroprevalence of leptospirosis in wild rats by MAT revealed 17.3% are positive for serovars icterohaemorrhagiae (12.3%), canicola (2.5%), ballum (1.2%), and pyrogenes (1.2%) (Mohamed Hassan *et al*, 2010). The findings from this animal study may suggest that serovars icterohaemorrhagiae and canicola infecting our subjects might have originated from rats, but this requires further studies.

Our study has a number of limitations. Firstly, the town service workers who participated in the study were predominantly male and all of them were of Malay race, which make our study unable to examine the effect of gender and race on the leptospirosis seropositivity. Secondly, we used IgM ELISA as a preliminary screening step to identify seropositive samples before proceeding to MAT to identify the specific serovar. The limited specificity and high sensitivity of IgM ELISA suggest that it would be more valuable as a 'rule-out test', with confirmation of positivity using the more specific MAT at a reference laboratory (Canal *et al*, 2013; Musso and La Scola, 2013). A number of participants might have had leptospiral infection in recent past and their IgM levels might have become undetectable, a situation which MAT could have excluded resulting in misclassification at the preliminary test stage. Thirdly, even though MAT is the reference method for the diagnosis and seroprevalence studies of leptospirosis, it has potential drawbacks similar to those of IgM ELISA, namely, misclassification of persons as seronegative and seropositive resulting in a potential bias. Fourthly, if certain *Leptospira* serovars were not included in the panel of live leptospires

that represent those present in the community (local serovars), it will lead to an incomplete panel resulting in false negative results (Plank and Dean, 2000; Musso and La Scola, 2013).

Leptospirosis is preventable if appropriate measures are taken, especially for modifiable risk factors. It is essential for people whose occupation involve exposure to leptospirosis to have knowledge of the disease, especially on prevention of infection. Education and awareness campaign specifically directed to the high risk groups and the community should be conducted. Workers in high risk occupation must wear appropriate personal protective apparels, such as boots, gloves, goggles, overall, face mask and eye protection based on their jobs and risks involved (Rao *et al*, 2003). Eradication of leptospires from the environment is obviously impractical, but measures to reduce direct contact with infected animals and indirect contact with urine contaminated soil and water remain the most effective strategy (Sharma and Yadav, 2008).

In conclusion, the present study highlights the high risk of leptospiral infection in town service workers in Kelantan, and identifies the predominant serovar as Sarawak, a poorly known strain endemic to Malaysia. This warrants further local study in human leptospirosis, particularly in other occupation high risk groups and in the community in general, and of animal leptospirosis to better understand local serovars and leptospirosis in Malaysia. Such information could help in developing better prevention and control measures of the disease locally and in other countries as well.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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