Leptospirosis and Workplace Environmental Risk Factors among Cattle Farmers in Northeastern Malaysia

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Abstract

Background: Leptospirosis is an emerging zoonosis and its occurrence has been reported to be rising globally. The environment plays an important role in the survival of *Leptospira* and determines the risk of infection. Those who were exposed to and had contact with contaminated environment through their occupational, recreational and other activities can be infected with the organism.

Objective: To determine the seroprevalence of leptospirosis among cattle farmers, prevalence of pathogenic *Leptospira*, and the workplace environmental risk factors for leptospirosis among cattle farmers in northeastern Malaysia.

Methods: A cross-sectional study involving 120 cattle farmers was conducted. The participants answered an interviewer-guided questionnaire that consisted of sociodemographic and workplace environment characteristics questionnaire, before having their blood sample taken for microscopic agglutination test (MAT). Seropositivity was determined using a cut-off titer of ≥1:100. 248 environmental samples were also collected from the cattle farms for polymerase chain reaction (PCR).

Results: The overall seroprevalence of leptospiral antibodies was 72.5% (95% CI 63.5% to 80.1%) and the prevalence of pathogenic *Leptospira* in the cattle farms environment was 12.1% (95% CI 8.4% to 17.0%). The independent factors associated with seropositivity of leptospirosis among cattle farmers were positive pathogenic Leptospira in the environment (Adj OR 5.90, 95% CI 1.34 to 26.01) and presence of garbage dumping in the farm (Adj OR 2.40, 95% CI 1.02 to 5.65).

Conclusion: Preventing leptospirosis incidence among cattle farmers necessitates changes in work environment. Identifying modifiable factors may also contribute to the reduction of infection.

Keywords: Leptospirosis; Environment; Risk factors; Agglutination test; Seroepidemiologic studies; Cattle

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Introduction

Leptospirosis, a disease caused by spirochetes of the genus *Leptospira*, is a globally re-emerging bacterial zoonotic disease that affects both humans and animals. It is transmitted to humans through contact between the skin or mucous membrane and water, moist soil, vegetation, or environmental surfaces contaminated with the urine of an infected animal. Given that human-to-human transmission of the disease is virtually unknown, human leptospirosis constitutes a dead-end infection, with the human as the dead-end host.[1,2]

Symptomatic leptospirosis usually manifests as a range of diseases, from a flu-like illness to Weil's syndrome resulting in multi-organ failure and pulmonary hemorrhage. The most severe form of the disease, the Weil's syndrome, is characterized by jaundice, renal failure, and hemorrhage with a variable clinical course; its case fatality rate ranges from 5% to 15%.[1] Leptospirosis is estimated to affect tens of millions of humans all over the world annually, with case fatality rates of 5% to 25%.[3] Among high-risk individuals, incidence may reach more than 100 per 100 000 people during outbreaks.[4] With a moderate annual incidence of 1 to 10 incidences per 100 000 people, the disease is considered endemic in Malaysia.[5]

Malaysia is characterized by a tropical climate and rainfall, thereby serving as a favorable environment for long-term bacterial survival. *Leptospira* can survive in moist, warm soil and in surface water for weeks to months, hence, a high incidence of leptospirosis.[6,7]

Agricultural workers are at a particularly high risk of contracting leptospirosis.[8,9] The livestock industry is one of the important agricultural sector in Malaysia. It provides the meats, milks and dairy products for the domestic use. According to 2016 statistics, it accounted for 11.6% of the total agricultural gross domestic product (GDP).[10]

The livestock industry in Malaysia comprises two main sectors, namely non-ruminant (swine and poultry) and ruminant (beef cattle, buffaloes, goats, and sheep). Currently, more than 90% of the ruminant sector in Malaysia is operated by small farm holders. These holders do not provide pastures for the animals, contrary to commercial and government farms, which have well-established infrastructure and pastures.[11] According to the Ministry of Agriculture (MOA) of Malaysia, the local cattle population was estimated to be 752 032 with 137 531 recorded slaughters in 2015. Beef production in the same year was 43 672 tons, amounting to a GDP of RM 1209.70 million (US$ 304.14 million).[12]

The objectives of this study were to determine the seroprevalence of leptospirosis among cattle farmers and the prevalence of pathogenic *Leptospira* in the cattle farms. Apart from that, this study was also conducted to identify the workplace environmental risk factors for leptospirosis among cattle farmers in northeastern Malaysia.

Materials and Methods

Participants and Study Design

In 2016, we conducted a cross-sectional study on six districts of northeastern Malaysia. The list of all cattle farmers available in those six districts was requested from the Department of Veterinary Services. A stratified random sampling method was used and the number of farmers selected for each district was proportionate to the total number of farmers in that district. The sample size for the study was calculated based on a seroprevalence of 37.5% of leptospirosis among animal handlers[13] and also the prevalence of 6.9% of pathogenic leptospires isolated from soil and water samples in Kelantan and Terengganu.[7] Assuming a type 1 error of 0.05 and 20% non-response rate and missing data, the minimum sample size was found to be 120 farmers and 265 environmental samples. As the number of the environmental samples had to be paired with the respondents, for 95 respondents two environmental samples and for another 25 respondents three environmental samples were taken. The selection was based on their farm's size. The inclusion criterion was cattle farmers who had worked for at least six months.

Blood Samples and Serological Test

All blood samples taken were sent to the Institute of Medical Research (IMR) for microscopic agglutination test (MAT). The MAT was conducted with a panel of live leptospire reference cultures, which were obtained from the Royal Tropical Institute (World Health Organization/Food and Agriculture Organization of the United Nations Collaborating Centre for Reference and Research on Leptospirosis) in Amsterdam for WHO serovars (Australis, Autumnalis, Bataviae, Canicola, Celledoni, Grippotyposa, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Hardjoprajitno, Patoc, Tarassovi, and Djasiman) and from the IMR for local serovars (Melaka, Terengganu, Sarawak, Lai, Hardjobovis, and Copenhagani).

Live leptospire cell suspensions that represent 20 serovars were added to serially diluted serum specimens in a well of microtiter plates and incubated at 30 °C for two hours. Agglutination was examined by dark-field microscopy. If the approximate number of free leptospires was <50% relative to the control well, then the sample was considered “positive.” We used a cut-off titer of ≥1:100, which is used in most laboratories for seroprevalence research to identify past exposure to the illness.[14,15]

Environmental Samples and Molecular Test

Water or suspensions of soil samples were collected from two or three places for each cattle farm. The best place in the farm was identified for the environmental sampling. According to set guidelines, environmental samples are preferably collected in the morning from damp or wet areas (puddles of water), shaded areas, between rocks and areas with presence of animal footprints.[16]

For soil samples, 20–30 g of soil were collected into 50 mL centrifuge tube using a sterile spatula, and were mixed with sterile water to keep it moist. The samples were then put into a box before being transported to the Universiti Sains Malaysia (USM) Microbiology Laboratory. The samples were suspended with sterile water of approximately three times the volume of the samples, and were then mixed by vigorous shaking. The suspension was allowed to settle for 5–7 minutes before filtered first through filter paper and then through 0.45-μm membranes. The filtered water was then inoculated into semisolid Ellinghausen and Mccullough, modified by Johnson and Harris (EMJH) culture media containing 200 μg/mL of 5-fluorouracil (5-FU).[17]

TAKE-HOME MESSAGE

- Leptospirosis is a globally re-emerging bacterial zoonotic disease that affects both humans and animals.
- Cattle farmers in northeastern Malaysia were at a high risk of leptospirosis. The dominant infecting serovar was Sarawak.
- The presence of pathogenic Leptospira and the presence of garbage dumping in the farms were significantly associated with leptospirosis seropositivity.
- Leptospirosis was transmitted indirectly from the cattle to farmers through the environment.

For the collection of water samples, 10–15 mL of water samples were taken from puddles, rivers, ponds, trenches, or swamps in the farm. The samples were put into a box to avoid exposure to sunlight prior to transportation to the USM Microbiology Laboratory. The samples were passed through a sterile 0.45-µm membrane filter, and 5–10 drops of filtrate were inoculated into semisolid EMJH culture media containing 200 µg/mL of 5-FU.[17] It was then incubated aerobically at room temperature. All the environmental samples were then sent to the IMR for polymerase chain reaction (PCR).

Ethics

Ethical approval was granted by the Research and Ethics Committee (Human), School of Medical Sciences, Health Campus, Universiti Sains Malaysia (USM/JEPeM/15050164). All of the farmers involved freely signed the informed written consent form. Guided by an interviewer, the respondents who agreed to participate in the research answered a questions in a data collection sheet about their sociodemographic and workplace environmental characteristics.

Statistical Analysis

Data were analyzed with the IBM Statistical Program for Social Sciences ver 22 for Windows®.[18] Confidentiality was maintained throughout the analyses. All continuous variables were expressed as mean and SD. Categorical variables were presented as frequencies and percentages. Variables with a p value <0.25 in univariate analysis were included in logistic regression analysis. A stepwise backward elimination method was used to identify the final logistic regression model of the association between work environment risk factors and leptospirosis seropositivity. The final model was checked for interactions and multicollinearity. A p value <0.05 was considered statistically significant.

Table 1: Sociodemographic characteristics of the respondents (n=120)

Variables	n (%)
Sex	
Male	104 (86.7)
Female	16 (13.3)
Marital status	
Married	94 (78.3)
Single/widower	26 (21.7)
Income, RM (US$)	
<1000 (<251)	73 (60.8)
1000–2000 (251–502)	32 (26.7)
>2000 (>502)	15 (12.5)
Education	
No formal education	12 (10.0)
Primary school	31 (25.8)
Secondary school	77 (64.2)

Results

Sociodemographics

All of respondents recruited were able to participate yielding a response rate of 100%. However, we were only able to collect 248 (93.6%) of 265 environmental samples calculated. The mean age of participants was 50.5 (SD 14.9, range 19 to 78) years (Table 1). All of them were Malays; most of them (78.3%) were married. The mean family size of the participants was 5.2 (SD 2.4) people. The majority of the respondents (90.0%) had either primary or secondary school education; only 12 (10.0%) had no formal education. Only 15 (12.5%) participants had monthly income of more than RM 2000 (US$ 502); majority of them (n=73, 60.8%) had less than

Table 2: Serovar distribution among seropositive cattle farmers (n=87)

Serovars tested*	n (%)
Sarawak	71 (59.2)
Patoc	25 (20.8)
Hardjobovis	8 (6.7)
Javanica	5 (4.2)
Tarrasovi	4 (3.3)
Grippotyphosa	3 (2.5)
Australis	2 (1.7)
Bataviae	2 (1.7)
Hardjoprajitno	2 (1.7)
Pyrogenes	2 (1.7)
Copenhageni	2 (1.7)
Pomona	1 (0.8)
Melaka	1 (0.8)
Terengganu	1 (0.8)
Lai	1 (0.8)

*Farmers tested may be positive for one or more serovars

RM 1000 (US$ 251) per month.

Seroprevalence of Leptospirosis among Cattle Farmers

The seroprevalence of leptospirosis among cattle farmers was 72.5% (95% CI 63.5% to 80.1%). In terms of serovar distribution among these seropositive cases, the tested sera most commonly reacted with the serovar Sarawak (59.2%) followed by serovar Patoc (20.8%) (Table 2).

Prevalence of Pathogenic *Leptospira* in the Environment

The prevalence of pathogenic *Leptospira* in the cattle farms environment was 12.1% (95% CI 8.4% to 17.0%). Only 20 soil and 10 water samples were found positive for pathogenic *Leptospira*. Two-thirds of the positive environment samples were from the soil.

Univariate Analysis

For the analysis, positive pathogenic *Leptospira* in environment is defined as any environmental samples in the farm that are found to be positive for pathogenic *Leptospira*. Univariate analysis showed that presence of positive pathogenic *Leptospira* and garbage dumping in the farm were significantly associated with leptospirosis seropositivity among cattle farmers (Table 3).

Multivariate Analysis

Binary logistic regression analysis revealed that presence of positive pathogenic *Leptospira* (Adj OR 4.15, 95% CI 1.15 to 14.99) and presence of garbage dumping in the farm (Adj OR 2.40, 95% CI 1.02 to 5.65) are independent predictors of seropositivity among cattle farmers.

The fitness of the preliminary model was validated. No interaction or multicollinearity was detected. Therefore, the model was accepted as the final model. The validation of the assumptions in the logistic regression showed that all the assumptions were supported by the data.

The fitness of the final model was then determined using the Hosmer-Lemeshow goodness-of-fit test. The model showed no significance (p=0.994), indicating fitness with a small discrepancy between observed and expected probabilities. Model fitness was also supported by the classification table and receiver operating characteristic (ROC) curve. The area under the ROC curve was 77.3% (95% CI 67.0% to 87.7%), indicating that the model can accurately discriminate 77.3% of the cases. Overall, the model correctly classified the cases at 72.5% accuracy. The satisfaction of these criteria indicates the fitness of the final model.

Table 3: Association between workplace environment risk factors with seropositive leptospirosis as determined by binary logistic regression (n=120)

Variables	Seropositive n=87, n (%)	Seronegative n=33, n (%)	Crude OR (95% CI)	Adj OR* (95% CI)
Positive pathogenic *Leptospira*				
No	61 (67)	30 (33)	1.00	1.00
Yes	26 (90)	3 (10)	4.26 (1.19 to 15.22)	4.15 (1.15 to 14.99)
Presence of river/trench/ pond/swamp				
No	17 (81)	4 (19)	1.00	—
Yes	70 (71)	29 (29)	0.57 (0.18 to 1.83)	—
Using river/trench/ pond/swamp				
No	44 (79)	12 (21)	1.00	1.00
Yes	43 (67)	21 (33)	0.56 (0.25 to 1.28)	0.45 (0.18 to 1.08)
Flooding				
No	31 (76)	10 (24)	1.00	—
Yes	56 (71)	23 (29)	0.79 (0.33 to 1.86)	—
Wading through stagnant water				
No	32 (78)	9 (22)	1.00	—
Yes	55 (70)	24 (30)	0.65 (0.27 to 1.56)	—
Garbage in farm				
No	39 (64)	22 (36)	1.00	1.00
Yes	48 (81)	11 (19)	2.46 (1.07 to 5.70)	2.40 (1.02 to 5.65)
Rats sighting in farm				
No	31 (69)	14 (31)	1.00	—
Yes	56 (75)	19 (35)	1.33 (0.59 to 3.02)	—

*Only three variables that attained a p<0.25 in univariate analysis were included in the multivariate analysis.

Discussion

The high seroprevalence of leptospirosis among the cattle farmers suggested that exposure to workplace environmental risk factors and *Leptospira* with different serovars may occur even though the respondents had developed the antibodies against a certain serovars from previous infection.[6,19]

The findings of the present study showed that the dominant infecting serovar was Sarawak. A limited number of studies have been devoted to *L. interrogans* serovar Sarawak, which is the local strain in Malaysia. A local study found that Sarawak is predominant in wild animals, especially squirrels and bats.[20] Information regarding pathogenicity and reservoir animals that harbor the serovar is minimal. In

For more information on work environment-related risk factors for leptospirosis among plantation workers in tropical countries see http://www.theijoem.com/ijoem/index.php/ijoem/article/view/699

the current work, we could not speculate on reservoirs of infection because animal surveys have not been carried out in this area. Further research on these local serovars can advance our understanding of infection sources and transmission routes, and contribute to the development of prevention programs.

According to our study, the prevalence of pathogenic *Leptospira* in the cattle farms in Kelantan was low; only 20 soil and 10 water samples were found positive for pathogenic *Leptospira*. However, the prevalence was higher that reported in a study done in 2010 by Ridzlan where he found 10 (6.9%) of 145 environmental samples were positive for pathogenic *Leptospira* by PCR. The environmental samples in that study were taken from the National Service Training Centre (NSTC) in Kelantan and Terengganu; the positive samples were more from water rather than soil samples.[7] Lack of animals at the NSTC could be the reason for the lower prevalence of pathogenic *Leptospira* in the 2010 study. Conversely, the presence of cattle in farms can lead to prolonged presence of the pathogen in the environment due to micturition by the cattle.

Cattle farmers are consistently exposed to risky environmental conditions by means of contact with fresh surface water and soil. With ideal environmental conditions, *Leptospira* can continue to survive for long periods in environments. An interesting finding was that *Leptospira* were able to survive for up to 43 days in soil flooded with rainwater. However, there are other environmental factors influencing the survival of *Leptospira*, for instance soil type, pH, temperature, and moisture.[21] Bejo reported in her study in 2001 that *L. interrogans* serovar Hardjo can survive for up to 264 hours (11 days) in rainwater, 72 hours (3 days) in diluted urine under Malaysian field conditions, and 984 hours (41 days) at 4 °C. These bacteria are also able to survive in chlorinated drinking water for up to 120 hours (5 days), but are killed immediately in seawater. In soil, the bacteria can survive for up to 144 hours (6 days).[22]

Leptospirosis seropositivity in cattle farmers was significantly associated with positive pathogenic *Leptospira* in the environment. The results of a binary logistic regression analysis showed that cattle farmers with positive pathogenic *Leptospira* in the farm's environment were approximately four times more likely to have leptospirosis seropositivity when compared to farms that were negative for pathogenic *Leptospira*. We therefore, concluded that leptospirosis was transmitted indirectly from the cattle to farmers through the environment.

The association between farmer's seropositivity and positive pathogenic *Leptospira* in the farm's environment could be due to the longer survival of pathogenic *Leptospira* in the environment from constant micturition of the cattle and other livestock. Higher pathogenic *Leptospira* concentration, will also lead to a greater risk of infection to farmers who come into contact with the contaminated environment. However, there are many other environmental criteria that support the survival of pathogenic *Leptospira* outside maintenance hosts including pH, temperature, soil type, water-retaining ability of soil, and presence of inhibitory agents not covered in our study.[23]

Among the respondents in the current research, 59 (49.2%) reported the presence of garbage dumping area in their farm. Garbage attracts rat species that are primary *Leptospira* reservoirs. The presence of such sites also contributes to the proliferation of rat colonies. These carrier animals feed, breed, and multiply in uncollected solid waste, rotting piles of garbage, and open dumping areas; thereby, posing a major health risk to humans that reside or work near these surroundings.[24,25] On top of this problem, domestic animals (*eg*, cattle, goats, and dogs) are also present at most open dumping sites, further increasing the likelihood of animal infection.

When adjusted for covariates, the farmers who reported the presence of garbage dumping in their farms exhibited an almost 2.5-fold increase in the likelihood of seropositivity compared with that for those working in farms with no garbage dumping. This result was supported by previous studies showing a significantly higher risk and seroprevalence of leptospirosis among workers involved in garbage collection and management.[26,27] Reservoir animals in cattle farm may contaminate surrounding areas with their urine containing leptospires. Similar to town service workers, cattle farmers may be infected through contact with a contaminated environment.

About half of the cattle farmers claimed to have used river, trench, pond, or swamp water available on their farm occasionally. This practice poses a risk for leptospirosis. Although it was not used as a primary water source, it was still used for swimming to cool down their bodies, bathing, washing their hands, feet, and work equipment, or watering their cattle. An epidemiological study of a leptospirosis outbreak in Sabah in 1999 indicated that the infection was contracted primarily by swimming in a creek that was most probably contaminated by the urine of infected animals from the surrounding area.[28] This factor however, was found significant neither in univariate nor in multivariate analyses in our study.

In conclusion, the seroprevalence results showed that cattle farmers were at a high risk of leptospiral infection. The presence of pathogenic *Leptospira* and the presence of garbage dumping in the farms were significantly associated with leptospirosis seropositivity. These findings pointed to the need to improve workplace environment condition to prevent leptospirosis incidence among cattle farmers in the future.

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References

1. Bharti AR, Nally JE, Ricaldi JN*, et al.* Leptospirosis: a zoonotic disease of global importance. *The Lancet Infect Dis* 2003;**3**:757-71.
2. Adler B, de la Peña Moctezuma A. Leptospira and leptospirosis. *Vet Microbiol* 2010;**140**:287-96.
3. Cachay ER, Vinetz JM. A global research agenda for leptospirosis. *J Postgrad Med* 2005;**51**:174-8.
4. World Health Organization. *Human Leptospirosis: Guidance For Diagnosis, Surveillance and Control*, Malta, WHO, **2003**.
5. Victoriano AFB, Smythe LD, Gloriani-Barzaga N*, et al.* Leptospirosis in the Asia Pacific region. *BMC Infectious Diseases* 2009;**9**:147-50.
6. Ganoza CA, Matthias MA, Collins-Richards D*, et al.* Determining risk for severe leptospirosis by molecular analysis of environmental surface waters for pathogenic Leptospira. *PLoS Med* 2006;**3**:308-10.
7. Ridzlan FR, Bahaman AR, Khairani-Bejo S, Mutalib AR. Detection of pathogenic Leptospira from selected environment in Kelantan and Terengganu, Malaysia. *Trop Biomed* 2010;**27**:632-8.
8. Brown PD, McKenzie M, Pinnock M, McGrowder D. Environmental risk factors associated with leptospirosis among butchers and their associates in Jamaica. *Int J Occup Environ Med* 2011;**2**:47-57.
9. Noor Rafizah AA, Aziah BD, Azwany YN*, et al.* A hospital-based study on seroprevalence of leptospirosis among febrile cases in northeastern Malaysia. *Int J Infect Dis* 2013;**17**:394-7.
10. Department of Statistic Malaysia. Selected Agricultural Indicators, Malaysia. In: Malaysia DoS, ed, **2017**.
11. Mohamed Z, Hosseini A, Kamarulzaman NH. Analy-

sis of Malaysian beef industry in Peninsular Malaysia under different importation policies scenarios and rate management systems. *Pertanika J Soc Sci & Hum* 2013;**21**:1-16.

12. MOA. Livestock. Agrofood Statistics 2015: Information Management and Statistics SectionPolicy and Strategic Planning Division, **2016**.
13. Sharma S, Vijayachari P, Sugunan AP, *et al.* Seroprevalence of leptospirosis among high-risk population of Andaman Islands, India. *Am J Trop Med Hyg* 2006;**74**:278-83.
14. Plank R, Dean D. Overview of the epidemiology, microbiology, and pathogenesis of Leptospira spp. in humans. *Microbes Infect* 2000;**2**:1265-76.
15. World Health Organization. Leptospirosis Laboratory Manual. WHO, **2007**.
16. Ministry of Health Malaysia. Guidelines For The Diagnosis,Management, Prevention And Control Of Leptospirosis In Malaysia, **2011**.
17. Hartskeel R, Smits H, Korver H*, et al. Instruction booklet of International course on laboratory methods for the diagnosis of leptospirosis.* 1st. KIT Royal Tropical Institute Publication **2004**:38-42.
18. IBM C. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp 2011.
19. Romero EC, Bernardo CCdM, Yasuda PH. Human leptospirosis: a twenty-nine-year serological study in São Paulo, Brazil. *Rev Ins Med Trop S Paulo* 2003;**45**:245-8.
20. Thayaparan S, Rpbertson I*,* Amraan F, *et al.* Serological Prevalence of Leptospiral Infection in Wildlife in Sarawak, Malaysia. *Borneo J Resour Sci Tech* 2013;**2**:71-4.
21. Levett PN. Leptospirosis. *Clin Microbiol Rev* 2001;**14**:296-326.
22. Bejo SK. Characterization of Leptospiral Isolates Obtained from Selected Cattle Farms in Malaysia. Dissertation, Universiti Putra Malaysia, **1997**.
23. Lau CL, Watson CH, Lowry JH*, et al.* Human leptospirosis infection in Fiji: an eco-epidemiological approach to identifying risk factors and environmental drivers for transmission. *PLoS Neglected Tropical Diseases* 2016;**10**. doi: 10.1371/journal.pntd.0004405
24. Walker A. Health and the environment. Waste disposal: fresh looks at a rotting problem. *BMJ* 1991;**303**:1391-5.
25. Herzstein JA, Bunn III WB. Solid Waste. *International Occupational and Environmental Medicine* 1992;**38**:620-32.
26. Solid waste. In: Herzstein JA, Bunn. WB, Fleming LE, Harrington JM, Jeyaratnam J, Gardner IR, editors. International. *Occupational and Environmental Medicine.* 1st ed. St. Louis: Mosby; **1998**, p. 620-32.
27. Shafei MN, Sulong MR, Yaacob NA*, et al.* Seroprevalence of Leptospirosis among Town Service Workers in Northeastern State of Malaysia. *International Journal of Collaborative Research on Internal Medicine & Public Health* 2012;**4**:395-403.
28. Tan DS. Leptospirosis in West Malaysia--epidemiology and laboratory diagnosis. *Malays J Pathol* 1979;**2**:1-6.
29. Koay TK, Nirmal S, Noitie L, Tan E. An epidemiological investigation of an outbreak of leptospirosis associated with swimming, Beaufort, Sabah. *Med J Malaysia* 2004;**59**:455-9.