Leptospirosis Occurrence in Agricultural Communities in Setiu, Terengganu

Nik Muhammad Hanif Nik Abdull Halim, Siti Rohana Mohd Yatim, Muhammad Afiq Zaki, Siti Nazrina Camalxaman

ABSTRACT--- Leptospirosis is a re-emerging infectious disease of public health importance. Infection can occur through occupational and environmental exposure of the causative agent through contaminated water in the agricultural field. In this study, soil and water samples were screened for the presence of Leptospira sp. In addition, the study aimed to explore the knowledge, attitudes and practice (KAP) on leptospirosis among agricultural communities in Setiu, Terengganu. A total of 40 environmental samples were randomly collected from rice fields, rubber plantation and oil palm plantation. All samples were examined for the presence of Leptospira sp by quantitative Real Time Polymerase Chain Reaction (qPCR) assay. KAP survey was carried out among 248 respondents using standardized modified translated questionnaires. Findings from this study revealed that only 5% of water collected from rice field area showed the presence of Leptospira sp. Similarly, only 5% of soil collected from oil palm plantation showed the presence of Leptospira sp DNA. Majority of respondents (52.58%) had moderate knowledge and unsatisfactory practice score (72.51%) but showed 75.21% of satisfactory attitude score. The findings also showed there is a significant difference in knowledge with regards to the signs and symptoms of leptospirosis (0.004). Furthermore, the practice score on rat control measures estimated 0.011 between three groups of respondents. The presence of Leptospira sp in the agricultural area and medium knowledge and practice among agricultural communities possesses environmental risks of contracting to leptospirosis. Hence, necessary interventions should be implemented by relevant authorities to prevent future transmission of leptospirosis in agricultural areas.

Index Terms:— Agriculture communities, KAP (Knowledge, Attitude and Practice), Leptospirosis, Real Time Polymerase Chain Reaction (qPCR).

I. INTRODUCTION

Leptospirosis is a zoonotic disease of global importance. It is endemic in tropical and subtropical countries in South-East Asia including Malaysia. Leptospirosis has been recognized as an emerging public health problem as evidenced by the escalating number of cases and outbreaks occurring worldwide [1]. The infection is caused by *Leptospira sp*, a pathogenic spirochete that can be disseminated through direct or indirect contact with the urine of infected animals. Under suitable conditions, *Leptospira sp* are able to survive in moist aqueous environments, which increases the chances of transmission following contact with contaminated water source [2].

Terengganu, a state situated in the east coast of Peninsular Malaysia has been listed as one of the five states with high leptospirosis cases in 2015. A total number of 461 documented reports, were reported and 8.00 per 100,000 population incidence rates was documented between the years 2004 - 2012 [3]. Leptospirosis cases are more likely to occur among occupational workers in the agricultural field, including farm workers, forestry-related workers as well as palm oil plantation workers, rice fields planter and rubber tapper compared to recreational exposure. To date, limited study exists on screening the presence of *Leptospira sp* in soil and water for agricultural area. Therefore, this study was conducted to provide evidence on the presence of *Leptospira sp* in the areas as well as to assess the level of knowledge, attitude and practice (KAP) among the agricultural communities.

II. MATERIALS AND METHODS

A. Soil and Water Sampling

A total of 40 samples were collected (20 soil and 20 water samples) from oil palm, rubber and rice field plantations in Setiu. 100 - 250ml of water samples were collected and subjected to laboratory investigations. Topsoil (15 cm by 5 cm) of approximately 200 g was collected from wet and shaded areas using spatula and was placed in sterile Whirl-Pak® bags. The temperatures and pH values of samples were measured. The samples were kept in the cool box and were transferred to the laboratory within 48 hours. Samples were stored at 4-8°C until further use, for not more than 3 days. The date, place of collection, and volume obtained for each sample was recorded.

B. Sample Filtration

Soil samples weighing 200–250 mg was mixed with sterile Phosphate-Buffered Saline (PBS) solution and allowed to settle for 15 to 20 min. Soil and 100 ml water samples was pre-filtered through sterile filter paper (Whatman no.1) and sieved using sterile 0.45µm and 0.22µm Millipore filters. The sedimentation obtained was suspended in PBS in Eppendorf® tubes. Centrifugation was performed at 5,000 × g for 10 min. The remaining sediment was suspended in 20–30µl PBS for subsequent DNA analysis.

Revised Manuscript Received on July 10, 2019.

Nik Muhammad Hanif Nik Abdull Halim, Centre of Environmental Health and Safety, Faculty of Health Sciences, Universiti Teknologi MARA Selangor, Puncak Alam Campus, 42300 Selangor, Malaysia.

Siti Rohana Mohd Yatim, Integrated Mosquito Research Group (I-MeRGe), Faculty of Health Sciences, Universiti Teknologi MARA Selangor, Puncak Alam Campus, 42300 Selangor, Malaysia.

Muhammad Afiq Zaki, Centre of Environmental Health and Safety, Faculty of Health Sciences, Universiti Teknologi MARA Selangor, Puncak Alam Campus, 42300 Selangor, Malaysia.

Siti Nazrina Camalxaman, Centre of Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA Selangor, Puncak Alam Campus, 42300 Selangor, Malaysia.

C. DNA Extraction

Total bacterial genomic DNA was extracted from the filtrate of water samples using a commercially available DNeasy Blood and Tissue Kit (Qiagen, USA). In addition, bacterial DNA from the soil samples filtrate was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, USA) following standard protocols.

D. Determination of DNA Concentration and Purity

The yield and purity estimation of extracted microbial DNA was assessed using an Eppendorf Biophotometer Plus (Eppendorf, Germany). UV absorbance values at 230 nm, 260 nm, and 280 nm were recorded prior to gene amplification

E. Leptospira sp Identification by qPCR

Soil and water samples were tested for the presence of Leptospira DNA using quantitative real-time PCR (qPCR). Reactions were performed in a final volume of 25 µL containing template DNA and microbial qPCR Master mix in a BioRad CFX 96 Real-Time PCR machine. The cycling conditions consisted of an initial denaturation at 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, annealing at 60 °C for 6 minutes followed by a final extension at 60°C for 6 minutes for 40 cycles. The threshold cycle (CT) was determined.

F. Knowledge, Attitude, and Practice Questionnaire

A set of validated translated questionnaire was developed based on previous KAP studies on leptospirosis by [4], [5]. The first section of the questionnaire focused on demographic questions. The second section evaluated respondents' knowledge on leptospirosis; its transmission, signs and symptoms, and methods of prevention based on a series of True/False/Unknown responses. Each correct answer was given 1 score, whereas a 0 score was given to wrong answers and the choice of 'I don't know'. The total knowledge score for each respondent will range from 0 to 10. Respondents who are unaware of leptospirosis were considered "poor knowledge". Respondents who scored less than (<72%) were considered to have "moderate knowledge", and those who scored more than (≥72%) where considered to have "good knowledge". The third section of the questionnaire addressed respondents' attitudes towards leptospirosis. A five-point Likert scale (1 = strongly disagree; 5 = strongly agree) was used to evaluate the participants' responses. The total attitude score for each respondent could range from 1 to 25. The final section of the questionnaire assessed respondents' self-reported prevention practices. It consisted of a five-level Likert scale question format (1 = never, 5 = always). The total practice score for each respondent ranging from 1 to 55. Total maximum scores for attitude and practice domain were 25 and 55 respectively. A score from zero to <75% may be considered unsatisfactory whereas a score of ≥75% to 100% may be taken as satisfactory attitude and practice score [6].

III. RESULTS AND ANALYSIS

A. Leptospira sp Identification by qPCR

The amplification of *Leptospira sp* DNA is illustrated in Fig. 1. The green line signifies Microbial DNA Positive Control (MPC). The blue line indicates Leptospira Positive Control (PPC) whereas the red line representing *Leptospira sp* DNA (tested samples). *Leptospira sp* DNA in both samples amplified moderately as compared to the MPC. Findings show that from a total 40 samples collected; 2 samples (5%) were positive for *Leptospira sp*. 1 sample (5%) was from the 20 water samples collected and another 1 (5%) was from the 20 soil samples collected. As shown in Fig. 2 the amplification of *Leptospira sp* from water sample obtained from rice field was observed at cycle 35. These findings indicate that only one water sample was infected with traces of *Leptospira sp* DNA. As for soil, out of 20 samples there was only one soil sample from the oil plantation that showed positive traces of *Leptospira sp* DNA. The amplification was observed at cycle 36 as shown in Fig. 3.

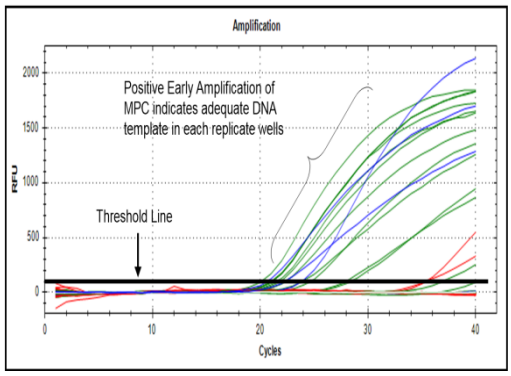

Fig. 1: Illustration showing amplification of *Leptospira sp* DNA (Samples), Microbial DNA Positive Control (MPC), and Leptospira Positive Control (PPC)

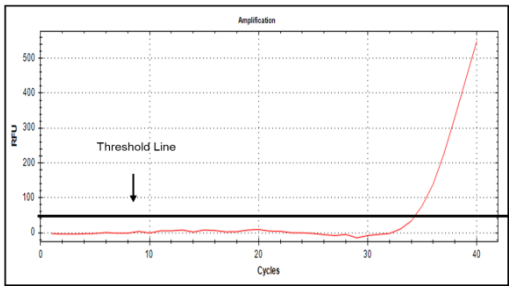

Fig. 2: Illustration showing amplification of *Leptospira sp* DNA in water sample obtain from rice field

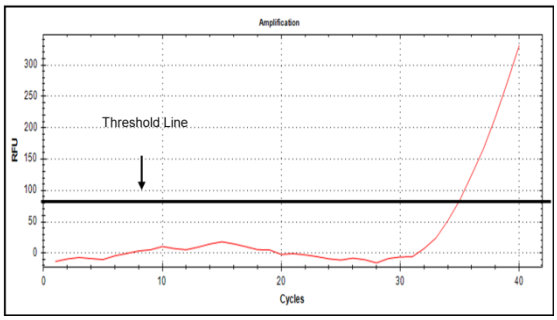

Fig. 3: Illustration showing amplification of *Leptospira sp* DNA in water sample obtain from oil plantation

Knowledge, Attitude and Practice (KAP) Scores Categorized by Occupation

Table 1 represents the KAP percentage scores for all respondents categorized by occupation. The corresponding mean value for knowledge was 52.78%; reflecting poor knowledge among respondents. The difference in their mean knowledge score was statistically significant at 0.05 level estimated at 0.044. There was also significant difference between this three groups of responses to questions on signs and symptoms (0.004) but not to the mode of transmissions and disease prevention questions at 0.05 level. In terms of attitude, the total mean attitude score for all respondents was 79.69%; signifying satisfactory attitude. The difference in their mean attitude score was not statistically significant at 0.05 level. In terms of prevention practices, the total mean practices score for all respondents were 72.51%; highlighting unsatisfactory practice. The difference in their mean practice score was not statistically significant at 0.05 level but there was a significant difference between this three groups of responses to questions on rat control measures estimated 0.011 but not for the use of protective gear and safety measures at 0.05 level.

Table 1: Knowledge, attitude and practice percentage scores and comparison categorized by occupation

	Oil Palm Farmer		Rice Fields Farmer		Rubber Tapper		All Respondents		
	n = 113		*n* = 73		*n* = 62		*n* = 248		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	*p*-Value
Total Knowledge Score	54.26	29.25	46.29	24.43	57.72	28.11	52.78	27.87	0.044*
Mode of Transmissions	69.41	13.75	67.28	12.65	71.10	13.95	69.19	13.51	0.263
Sign and Symptoms	50.15	36.22	34.70	26.61	48.92	30.46	45.30	32.83	0.004*
Disease Prevention	61.80	39.13	52.74	36.64	64.78	36.97	59.87	38.03	0.143
Total Attitude Score	77.88	12.78	81.97	14.61	80.32	11.20	79.69	13.06	0.102
Total Practice Score	71.20	16.87	71.73	12.79	75.84	12.11	72.51	14.72	0.118
Protectives Gears and Safety Measures	69.41	13.75	67.28	12.65	71.06	13.95	69.19	13.51	0.263
Rat Control Measures	74.33	25.25	79.52	15.99	84.19	16.72	78.33	21.19	0.011*

IV. DISCUSSION & RESULTS

A. Leptospira sp Identification by qPCR

It was clearly observed that *Leptospira sp* DNA in both positive sample were amplified moderately as compared to the MPC. This is due to the fact that the traces of *Leptospira sp* DNA were present in both sample but only in small quantity. Despite, a low quantity of DNA presence in both water and soil samples, the amplification of *Leptospira sp* DNA was confirmed and valid as all of the MPC and PPC were successfully amplified.

The only 2 positive samples consist of 1 (5%) of 20 water samples and 1 (5%) of 20 soil samples collected. The present study results similar to the results of [7] who are isolated *Leptospira sp* 5-10% from the total number of water samples from recreational area. The results of presented study are also similar to those recorded by [8] who found *Leptospira sp* in 5% water samples collected in flooding area. The occurrence noted in this current study is lower compared to those obtained by [9] noted the presence of 23.1% *Leptospira sp* in water samples and in [10] found that 23.1% and 23.3% presence of *Leptospira sp* in water and soil sample that have been collected from east coast state in Malaysia respectively.

The occurrence of *Leptospira sp* in both water and soil samples might be due to infestation of rat and other *Leptospira sp* host. The transmissions of *Leptospira sp* in the environment could be due to direct contact with urine or body fluids from contaminated animals or host through direct and indirect contact to the soil and water by *Leptospira sp* [11]. It is well known that mammalian like rodents excrete *Leptospira sp* in their urine and make as reservoirs for leptospirosis transmission. These bacteria are maintained in domestic environments especially in water and soil by transmission among rodent species [12]. *Leptospira sp* can survive for weeks or up to months in wet soil on dry days and appeared in the surface water on the rainy days hence these condition showed that soil could be the reservoir of *Leptospira sp* in environment [13], [14]. In [15] previously reported that *Leptospira sp* are most frequently associated with soils of high moisture and organic matter content. It was suggested that the moisture content ≥20% were found to be more positive *Leptospira sp* in environmental sample [13].

A good condition of the environments is associated with appropriate factors such as pH, temperatures, characteristics of environment and availability of host act as a reservoir would maintain the presence of *Leptospira sp* [10]. In this presented study, the positive samples were found in both rice fields and oil palm plantations. Environment of agricultural land provides very ideal habitat for the rodents as main reservoirs to transfer the *Leptospira sp*. This location has wet environment, with large rodent population and numerous animals cohabit and leading to high exposure to workers whom without or with minimum protection to get infected. The climatic conditions in this country such as warm water, heavy rainfall, and high humidity provide an appropriate medium for the organism to survive in environment [9]. Another suitable conditions like stagnant water, depth ranging from 5 to 10 cm, median pH is 7.6 and median temperature 34.5°C which favour the survival of

Leptospira sp [10]. Higher concentration of *Leptospira sp* was found in stagnant water rather than in rainwater or underground water [16]. Puddles of water in rice fields during rice grow is an optimal condition that promote the growth of *Leptospira sp* bacteria. Rice fields and oil palm plantations also have abundance of food resources such as paddy and oil palm fruits or remnant of planter food which attract a lot of rodents to forage. This attracts rodents to populate and become potential reservoirs for *Leptospira sp*. Besides rodents, other factors that can support the transmission and growth of *Leptospira sp* are found fields, bushes or wet grass also can be a bacteria sources [17].

B. Knowledge, Attitude and Practice (KAP) Scores Categorized by Occupation

With respect to the sign and symptoms, the knowledge score among respondents was relatively poor. The findings only 45.3% of the respondents answered that the infected person may have certain sign and symptoms. This results also similar among respondents from Philippines and Trinidad with 44% and 47% respectively [5], [18] but lower compared to Brazil with 68% [4]. This fact indicates that majority of people are not aware of the disease as most of infected persons are asymptomatic. Knowledge of the signs and symptoms as well as complications among the respondents are indeed crucial because it will lead them to recognize the danger of leptospirosis at early stage of infection. This can help them to know proper management which eventually saves their lives. The level of knowledge on mode of transmission and causes contributing to leptospirosis noted in this current study is better than the other factors. This finding is quite lower compared to those obtained by [4], [5], [19], [18]. This may imply that the respondents know the disease is caused by bacteria and it is related to rodents and its urine and how the microbes enter into the body. With regards to disease prevention, our study findings showed 60% from total respondents answered correctly. This is also lower compared to [4], [5], [19] respectively. Indeed, without knowing how to prevent from being infected by leptospirosis, we cannot expect that respondents to be aware of the disease and it is almost impossible for them to be motivated in adopting good preventive measures in their working practices. It is interesting that majority of respondents who had ever know that leptospirosis can be cured with antibiotics. This misbelief may spread in the community and may finally contribute to poor management in treating leptospirosis. This issue is very crucial and needs to emphasized to them.

In terms of attitude about leptospirosis, the total mean attitude score for all respondents were 79.69%. Thus, they were considered to have satisfactory attitude. These study findings showed that majority of respondents had positive attitude and this comparable with the proportion registered by [5] from Philippines found that the higher level of attitude among respondents compared to the study conducted by [4] from Brazil and [18] from Trinidad. The low level of awareness and attitude of leptospirosis makes it a disease of public health importance. On the other hand, other study by [19] from Philippines presented medium attitude and concern on leptospirosis. This is a good starting to implement prevention and control programs because there still difficulties in convincing community to take all the needed safety precautions even though they may be well informed on the leptospirosis. Our suggestion is that the attitude towards the prevention and taking action in reducing infection and to believe that medicine can treat leptospirosis in their attitude need to be corrected.

In terms of prevention practices related to leptospirosis, the total mean practices score for all respondents were 72.51%. Thus the respondents showed unsatisfactory practice. The present results also similar with the study by [5] from Philippines reported that respondents take action to avoid from being infected by the disease This is not comparable to the [19] from [18] also from Trinidad showed that there was a positive attitude towards general health and good sanitary practices among the population. They are also maintained the cleanliness in the surrounding and keep up good hygiene. On the other hand, in [5] from [4] from Brazil also found that respondents who performed risk activities had a limited access to protective gears and safety measures such as boots and gloves. In terms of prevention from rat infestation, they found that almost all respondents performed activity control to prevent rat from enter to the household.

From the results, it was probably because they realized the benefits of practising good behaviour. It is possible that the information from mass media and from health services were sufficient to address their lack of understanding in disease prevention. Respondents are particularly weak in the protective gears and safety measures or “PPE” sub category. Items in this sections revealed a lower mean from respondents who practiced wearing proper boots and gloves while working in muddy water or damp soil as well as wade in the flood water. PPE are important and remained the main predictors for Leptospira infection [20]. Respondents may not appreciate the importance of such practice as disease preventions because they simply lack of general knowledge on leptospirosis and the preventions against it. Future health education and promotion action should be implement to tackle this issue as well

V. CONCLUSION

Leptospira sp were detected in samples collected from both oil palm plantation and rice fields. This pose health risks from direct contact of contaminated water and soil among agricultural communities. Findings from this study also revealed variation in the knowledge and attitude of agricultural communities regarding preventive and protective measures against leptospirosis. Despite positive attitude and medium practice score, most of the respondents scored low level of knowledge in reference to the signs and symptoms of leptospirosis. Further

control and preventive strategies including targeted education and awareness campaigns are necessary to hinder future dissemination of the aetiological agent.

ACKNOWLEDGMENT

The authors gratefully to thank the Setiu District Health Office and staffs for supporting this project. Authors do not possess any conflict of interest.

REFERENCES

1. R. A. Hartskeerl, M. Collares-Pereira, and W. A. Ellis, "Emergence, control and re-emerging leptospirosis: Dynamics of infection in the changing world," Clinical Microbiology and Infection, 17(4), 2011, pp. 494-501.
2. C. Muñoz-Zanzi, M. Mason, C. Encina, A. Astroza, and A. Romero, "Leptospira contamination in household and environmental water in rural communities in southern Chile," International Journal of Environmental Research and Public Health, 11(7), 2014, pp. 6666-6680.
3. D. Benacer, K. L. Thong, N. C. Min, K. B. Verasahib, R. L. Galloway, R. A. Hartskeerl, M. Souris, and S. N. M. Zain, "Epidemiology of human leptospirosis in Malaysia, 2004–2012," Acta Tropica, 157, 2016, pp. 162-168.
4. W. N. de Araújo, B. Finkmoore, G. S. Ribeiro, R. B. Reis, R. D. M. Felzemburgh, J. E. Hagan, M. G. Reis, A. I. Ko, and F. Costa, "Knowledge, attitudes, and practices related to Leptospirosis among urban slum residents in Brazil," American Journal of Tropical Medicine and Hygiene, 88(2), 2013, pp. 359-363.
5. J. Arbiol, P. Orencio, H. Nomura, Y. Takahashi, and M. Yabe, "Knowledge, attitude and practices towards leptospirosis among lakeshore communities of Calamba and Los Baños, Laguna, Philippines," Agriculture, 6(2), 2016, pp. 1–12.
6. Z. M. Azfar, S. M. Nazri, A. M. Rusli, O. Maizurah, W. M. Zahiruddin, Y. N. Azwany, I. Nabilah, H. S. Asma, and B. D. Aziah, "Knowledge, attitude and practice about leptospirosis prevention among town service workers in northeastern Malaysia: A cross sectional study," Journal of preventive medicine and hygiene, 59(1), 2018, pp. 92–98.
7. S. Ismail, N. Z. A. Wahab, N. Badya, N. I. A. Rahman, C. C. Yeo, A. Z. A. Latif, and M. Haque, "A study on the presence of pathogenic Leptospira spp. in environmental water samples obtained from selected recreational areas in Terengganu, Malaysia," Research Journal of Pharmacy and Technology, 7(10), 2014, pp. 1153–1157.
8. A. W. Fatla, V. Zajac, B. Wasinski, J. Sroka, E. Cisak, A. Sawczyn, and J. Dutkiewicz, "Occurrence of Leptospira DNA in water and soil samples collected in eastern Poland," Annals of Agricultural and Environmental Medicine, 21(4), 2014, pp. 730–732.
9. D. Benacer, P. Y. Woh, S. N. M. Zain, F. Amran, and K. L. Thong, "Pathogenic and saprophytic Leptospira species in water and soils from selected urban sites in Peninsular Malaysia," Microbes and Environments, 28(1), 2013, pp. 135–40.
10. F. R. Ridzlan, A. R. Bahaman, S. Khairani-Bejo, and A. R. Mutalib, "Detection of pathogenic Leptospira from selected environment in Kelantan and Terengganu, Malaysia," Tropical Biomedicine, 27(3), 2010, pp. 632-638.
11. M. A. Matthias, J. N. Ricaldi, M. Cespedes, M. M. Diaz, R. L. Galloway, M. Saito, A. G. Steigerwalt, K. P. Patra, C. V. Ore, E. Gotuzzo, and R. H. Gilman, "Human leptospirosis caused by a new, antigenically unique Leptospira associated with a Rattus species reservoir in the Peruvian Amazon," PLoS Neglected Tropical Diseases, 2(4), 2008, pp. 1-12.
12. A. I. Ko, C. Goarant, and M. Picardeau, "Leptospira: The dawn of the molecular genetics era for an emerging zoonotic pathogen," Nature Reviews Microbiology, 7(10), 2009, pp. 736–747.
13. M. Saito, S. Y. A. M. Villanueva, A. Chakraborty, S. Miyahara, T. Segawa, T. Asoh, R. Ozuru, N. G. Gloriani, Y. Yanagihara, and S. Yoshida, "Comparative analysis of Leptospira strains isolated from environmental soil and water in the Philippines and Japan," Applied and Environmental Microbiology, 79(2), 2013, pp. 601–609.
14. J. Donyaee, "Evaluation of the surface water polluted with pathogenic Leptospirosis in Mazandaran Province (North of Iran) at 2014," Evaluation, 1(1), 2014, pp. 14–17.
15. R. A. Henry, and R. C. Johnson, "Distribution of the genus Leptospira in soil and water," Applied and Environmental Microbiology, 35(3), 1978, pp. 492–499.
16. C. F. Pui, L. M. Bilung, K. Apun, and L. Su'ut, "Diversity of Leptospira spp. in rats and environment from urban areas of Sarawak, Malaysia," Journal of Tropical Medicine, 2017, 2017, pp. 1-8.
17. H. Sumanta, T. Wibawa, S. Hadisusanto, A. Nuryati, and H. Kusnanto, "Spatial analysis of Leptospira in rats, water and soil in Bantul district Yogyakarta Indonesia," Open Journal of Epidemiology, 5(1), 2015, pp. 22–31.
18. A. R. Mohan, and D. D. Chadee, "Knowledge, attitudes and practices of Trinidadian households regarding leptospirosis and related matters," International Health, 3(2), 2011, pp. 131-137.
19. C. R. Quina, J. U. Almazan, and J. B. Tagarino, "Knowledge, attitudes, and practices of leptospirosis in Catbalogan City, Samar, Philippines," American Journal of Public Health Research, 2(3), 2014, pp. 91–98.
20. M. A. S. Johnson, H. Smith, P. Joseph, R. H. Gilman, C. T. Bautista, K. J. Campos, M. Cespedes, P. Klatsky, C. Vidal, H. Terry, and M. M. Calderon, "Environmental exposure and leptospirosis, Peru," Emerging Infectious Diseases, 10(6), 2004, pp. 1016–1022.