

## Detection of *Leptospira* spp. using polymerase chain reaction technique from kidney of *Rattus norvegicus* from Grenada, West Indies

Bhumika Sharma, Katelyn Thille, Nia Rametta and Ravindra Sharma

Department of Pathobiology, School of Veterinary Medicine, St. George's University, Grenada, West Indies.

**Corresponding author:** Bhumika Sharma, e-mail: [bsharma@sgu.edu](mailto:bsharma@sgu.edu)

**Co-authors:** KT: [kthille@sgu.edu](mailto:kthille@sgu.edu), NR: [nrametta@sgu.edu](mailto:nrametta@sgu.edu), RS: [rsharma@sgu.edu](mailto:rsharma@sgu.edu)

**Received:** 21-05-2019, **Accepted:** 13-08-2019, **Published online:** 24-09-2019

**doi:** 10.14202/IJOH.2019.81-85 **How to cite this article:** Sharma B, Thille K, Rametta N, Sharma R. Detection of *Leptospira* spp. using polymerase chain reaction technique from kidney of *Rattus norvegicus* from Grenada, West Indies. Int J One Health 2019;5:81-85.

### Abstract

**Aim:** This study aimed to find out the prevalence of active infection of *Leptospira* spp. in *Rattus norvegicus* from Grenada, West Indies, through polymerase chain reaction (PCR).

**Materials and Methods:** One hundred and forty-nine rats were trapped, anesthetized and their kidneys collected aseptically. DNA was extracted from the kidney tissue of each rat. PCR was performed targeting *LipL32* gene. Eighteen PCR-positive amplicons for *LipL32* gene segment were purified and sent for direct sequencing to the sequencing facility of MCLAB (South San Francisco, USA). Results of sequencing were read and interpreted. The prevalence of *Leptospira* spp. in relation to sex and age was also recorded.

**Results:** All amplified sequences were compared to the sequences present in GenBank using basic local alignment search tool (BLAST) from the online website National Center for Biotechnology Information, the results revealed that six samples had similarity to *Leptospira interrogans* strain 1399/2016 and eight samples had similarity with *Leptospira borgpetersenii* serovar Hardjo-bovis strain L49. Of 149 kidney samples, only 14 were positive for *Leptospira* spp. by PCR giving an incidence of 9.3%. There was no significant difference found in relation to sex and age.

**Conclusion:** This is the first report confirming active infection of *Leptospira* spp. in *Rattus norvegicus* in Grenada using PCR. The presence of active infection in rats can be considered as high risk for humans. Further research to understand the epidemiology of leptospirosis in Grenada is suggested.

**Keywords:** *Rattus norvegicus*, Grenada, kidney, *Leptospira* spp., polymerase chain reaction.

### Introduction

Leptospirosis, a reemerging zoonotic disease of animals and humans, is caused by *Leptospira* spp. of spirochete bacteria. Transmission of *Leptospira* spp. to susceptible hosts occurs by ingestion of water and food contaminated with urine and tissues of infected animals. Another mode of transmission is through cut or abrasion in the skin. After ingestion or skin cut, bacteria circulate in the body and finally localize in proximal convoluted tubules of the kidney where these are protected from antibiotics and other blood defense mechanisms and are excreted chronically in the urine [1].

Of several diagnostic methods, the two commonly used serological tests are the microscopic agglutination test (MAT) and the enzyme-linked immunosorbent assay (ELISA). MAT is considered as the “gold standard” test for leptospirosis, which detects both IgG and IgM. MAT is highly specific and

generally used in reference laboratories as it requires propagation of live *Leptospira* strains. MAT can detect infecting serovars, but it has a low sensitivity [2]. The ELISA detects IgG and IgM antibodies separately and is highly sensitive [2]. Other serological tests including MAT and ELISA are insensitive during the early phase of the disease. The need for rapid diagnosis in the early phase of infection led to the development of numerous polymerase chain reaction (PCR) assays. PCR is based on the molecular detection of amplified bacterial genes fragments present in host species. Examples of some of the target genes used for *Leptospira* PCR are *gyrB* and *rrs* (*16S rRNA* gene) or genes present in pathogenic *Leptospira* spp. as *LipL32*, *Ifb1*, *Lig A*, and *LigB2* [3-5]. Real-time quantitative PCR (qPCR) has replaced conventional PCR, as in later diagnostic value was unclear. Several qPCR methods targeting *LipL32* and *rrs* gene for simultaneous detection of pathogenic and non-pathogenic leptospires have been recently developed [4,5].

*Rattus norvegicus* is considered a major reservoir of leptospires and a source of *Leptospira* infection to humans and animals. In the Caribbean, infection of *Leptospira* spp. has been documented in humans and animals [6]. The first report of *Leptospira* spp. in Grenada was published in 1985 [7], in which MAT-based serological analysis of samples from

Copyright: Sharma, et al. This article is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

cattle, pigs, sheep, goats, horses, donkeys, and chickens had *Leptospira icterohaemorrhagiae* as the main serovar along with others. There are few published reports on *Leptospira* spp. exposure of *R. norvegicus* in the Caribbean. Peters *et al.* [8] while reviewing the serovars of *Leptospira* spp. in the Caribbean reported *L. icterohaemorrhagiae* serovar Copenhageni and *L. icterohaemorrhagiae* serovar Icterohaemorrhagiae in rats in Grenada. They also reported serovar Icterohaemorrhagiae in rats in other Caribbean nations (Barbados, Guadeloupe, Jamaica, Trinidad, and Tobago). In Grenada, for the first time, Keenan *et al.* [9] reported 27% seroprevalence of antibodies to *Leptospira* spp. in *R. norvegicus*, using MAT and ELISA techniques. They found *Leptospira icterohaemorrhagiae* serogroup with greatest frequency. In another recent survey conducted in *R. norvegicus*, Sharma *et al.* [10] found higher seroprevalence (45.5%) in Grenada. Since they used ELISA, serogroups were not identified. Until now, no molecular techniques have been used to identify *Leptospira* spp. serovars in *R. norvegicus* in Grenada.

This study aimed to identify the prevalence of pathogenic serovars of *Leptospira* spp. in kidney of *R. norvegicus* from Grenada using PCR technique.

## Materials and Methods

### Ethical approval

The project (Detection of Zoonotic Pathogens in *Rattus norvegicus* in Grenada) was approved by the Institutional Animal Care and Use Committee (IACUC #16009-R) of St. George's University, Grenada.

### Study area

Grenada is the southernmost country in the Caribbean Sea with an area of 348.5 km<sup>2</sup>. The country with low hills, small trees, shrubs, and tropical climate is most suitable for rats. The country is comprised of six parishes: St. Patrick, St. Mark, St. Andrew, St. John, St. George, and St. David. St. David and St. George parishes, which have a higher human population compared to the other four parishes were selected for the study.

### Collection of rats

One hundred and forty-nine rats were collected live from May 1 to July 14, 2017, using traps (45 cm l × 15 cm w × 15 cm h) with cheese and various local fruits as bait. Attempts were made to trap the rats from and near the residential buildings. Traps were placed 2 days per week in the evening and visited the morning of the next day. Traps with rats were covered with black cloth and transported to the necropsy laboratory of St. George's University, School of Veterinary Medicine. Rats were anesthetized using 1–2% isoflurane in oxygen through portable vet anesthesia machine isoflurane vaporizer VET CE, manufacturer DRE (Avante Health Solution Company, USA).

### Collection of samples and testing

The anesthetized rats were examined for their physical health and weighed. Sex and weight were

recorded. Rats below 100 g were grouped as young and those over 100 g as adult, following the methodology used by Panti-May *et al.* [11]. Blood was collected from the heart through the thoracic wall and rats were exsanguinated this way. The abdominal cavity of each rat was opened using a new surgical blade and forceps. Kidneys were examined for any gross abnormalities and recorded. Kidneys were aseptically removed and stored at –80°C in sterile tubes until tested by PCR.

### DNA extraction

One hundred and forty-nine kidney samples from trapped *R. norvegicus* were processed for DNA extraction. Approximately 25 mg of kidney tissue sample was pulverized using a separate sterile disposable scalpel blade for each rat. Four hundred microliters of phosphate buffer saline were then added to the macerated kidney samples in a microcentrifuge tube and further homogenized using 10–12 silica beads and beating in a bead beater machine for 30 s at pulse. Forty microliters of proteinase-K and 360 µL of tissue lysis buffer (ATL) were added to the samples and incubated at 56°C for overnight digestion. The samples were then processed using the DNeasy blood and tissue kit (QIAGEN, Valencia, CA, USA) according to manufacturer's instruction. The final elution for each sample was 200 µL in buffer AE provided in the DNeasy blood and tissue kit. Each of the DNA sample was quantified spectrophotometrically using Thermo Scientific™ NanoDrop 2000 (Waltham, MA USA). The extracted DNA was stored at –20°C until PCR was performed.

### Polymerase chain reaction

A single-step PCR using the primer pair LipL32-45F (5'-AAG CAT TAC CGC TTG TGG TG-3') and LipL32-286R (5'-GAA CTC CCA TTT CAG CGA TT-3') following Stoddard *et al.* [12] was performed to amplify a 242 bp fragment of an outer membrane lipoprotein present in the pathogenic *Leptospira* spp. [12–14]. Nuclease-free water was used as a negative control and genomic DNA from *L. interrogans* serovar Copenhageni (ATCC® BAA-1198D-5™) served as a positive control. Each PCR amplification was carried out using a 25 µl reaction mixture containing a final concentration of 1× PCR reaction buffer, 1.25 U of Taq DNA polymerase, 1.5 mM MgCl<sub>2</sub>, 0.2 mM each of dNTP mixture, with 0.5 µM of each primer, and 3 µL (~5 ng–10 ng) of DNA template. The cycling conditions were as follows: Initial denaturation at 94°C for 5 min followed by 40 cycles of 1 min denaturation at 94°C, 1 min of annealing at 63°C, and 45 s of extension at 72°C, and a final 5 min extension at 72°C after the last cycle.

Twenty microliters of the PCR products were subjected to electrophoresis with 1.5% agarose gel, stained with ethidium bromide, and photographed under gel documentation system (LabNet International Inc., Edison, NJ, USA). Amplicons were purified using QIAquick Gel Extract Kit (Qiagen, Valencia,

CA, USA) following manufacturer's instructions and sent for sequencing to the sequencing facility provided by MCLAB (South San Francisco, CA, USA).

#### Statistical analysis

Data were analyzed using a Chi-square analysis and stratified by gender, age, and parish of rats in Microsoft Excel 2017 software (Redmond, Washington, U.S.A.). Statistical significance was set at  $p=0.05$ .

#### Results

Kidney samples of rats under study did not show any gross abnormality. Genomic DNA from 149 rat kidney samples was used to perform PCR to amplify DNA fragments *LipL32* gene. An approximately 242 bp PCR product for *LipL32* gene was amplified from the kidney samples of 21 rats (14%). Eighteen PCR-positive amplicons for *LipL32* gene were purified and sent off for sequencing. Partial sequences for *LipL32* gene from 14 samples were submitted to GenBank® (Table-1). The *LipL32* gene sequences showed 90–100% homology to one another. All the sequences were compared to the sequences present in GenBank® using BLAST from the online website, the National Center for Biotechnology Information. The results from *LipL32* gene sequences revealed that six samples had highest similarity (between 96.53% and 100%) to *L. interrogans* strain 1399/2016 (accession number MF663255.1) and eight samples

had highest similarity (between 99.01% and 100%) with *L. borgpetersenii* serovar Hardjo-bovis strain L49 (accession number CP033440.1) as recorded in Table-1. The result of PCR in relation to parish, age, and sex of rats is presented in Table-2. The relation between *L. interrogans* serovar Copenhageni and *L. borgpetersenii* serovar Hardjo-bovis strain was not significant ( $p=0.71$ ). There was no statistical significance in the prevalence of *Leptospira* spp. between St. David and St. George parish ( $p=0.169$ ). The prevalence of infection in relation to age was not significant ( $p=0.26$ ).

#### Discussion

The use of PCR over serological diagnostic tests for leptospirosis is being recommended, due to its ability to distinguish between pathogenic and non-pathogenic *Leptospira* serovars [15]. PCR is also of value in the early phase of the disease where serological tests are not reliable. The previous researchers have used number of gene sequences in their research for conventional PCR and qPCR such as *23S rRNA* [15,16]; *GyrB* [17]; *flab* [18]; *16S rRNA* [19]; and *LigA/B* [15]. Xue *et al.* [20] reported the presence of these genes in pathogenic *Leptospira* spp. In the present study, *LipL32* gene was used, which has been used in PCR by the previous researchers [21-24].

Of 149 kidney samples tested from *R. norvegicus*, 14 (9.3%) were positive by PCR for *LipL32* gene.

**Table-1:** New sequences submitted to the GenBank® database for *LipL32* gene sequence.

Sample ID	Accession No.	Sequence length (bp)	Similarity to <i>L. interrogans</i> strain 1399/2016; Accession # MF663255.1 (%)	Similarity to <i>L. borgpetersenii</i> serovar Hardjo-bovis strain L49; Accession # CP033440.1 (%)
RK27	MK895960	202	99.01	
RK69	MK895961	202		99.01
RK147	MK895962	202	96.53	
RK163	MK895963	202		100.00
RK1	MK895964	202	100.00	
RK12	MK895965	202	100.00	
RK85	MK895966	202		100.00
RK139	MK895967	202		100.00
RK158	MK895968	202		100.00
RK162	MK895969	202		100.00
RK165	MK895970	202	100.00	
RK166	MK895971	202	97.03	
RK167	MK895972	202		100.00
RK170	MK895973	202		100.00

*L. interrogans*=*Leptospira interrogans*, *L. borgpetersenii*=*Leptospira borgpetersenii*

**Table-2:** Distribution of *Leptospira* serovars targeting *LipL32* gene in *R. norvegicus* in Grenada.

Serovars	Parish		Total positive	Sex		Age	
	St. George	St. David		M	F	Adults	Juvenile
<i>L. interrogans</i> Copenhageni/ <i>Icterohaemorrhagiae</i>	1	5	6/14 (42.8%)	4	3	4	2
<i>L. interrogans</i> / <i>L. borgpetersenii</i> Hardjo-bovis strain	3	5	8/14 (57.2%)	3	4	5	3
Total	4/149 (2.6%)	10/149 (6%)		7/14 (50.0%)	7/14 (50.0%)	9/14 (64.2%)	5/14 (35.8%)

*L. interrogans*=*Leptospira interrogans*, *L. borgpetersenii*=*Leptospira borgpetersenii*

The results from *LipL32* gene sequence revealed 6 samples (42.8%) in similarity with *L. interrogans* serovar Copenhageni and 8 samples (57.2%) in similarity with *L. borgpetersenii* serovar Hardjo-bovis strain L42. Our results of serovar distribution in *R. norvegicus* are similar to Cosson *et al.* [23], who reported 46% *L. borgpetersenii* and 36% *L. interrogans* in rodents in Southeast Asia. In Kaula Lumpur, the ratio of serovars reported by Benacer *et al.* [25] coincides with our results. They also reported higher prevalence of *L. borgpetersenii* serovar Javanica, (85%) than 35% *L. interrogans* serovar Bataviae. Both these *Leptospira* spp. are known to be pathogenic. The prevalence of *Leptospira* spp. (7.0%) observed by Cosson *et al.* [23] in PCR was similar to our results. This indicates a wide prevalence of *L. interrogans* and *L. borgpetersenii* species in rodents. Loan *et al.* [24] used *LipL32* gene in RT-PCR and reported 5.8% prevalence of *Leptospira* spp. in rats in Vietnam, which is slightly lower than our findings. Previous studies found different levels of infection in *R. norvegicus* using various gene-targets in PCR, for example 11.6% in Cambodia [26]; and 6.7% in Malaysia [25]. However, in Southeast China the infection rate was found to be between 20–40%, depending on the RT-PCR methods used [27]. The variation in the prevalence of *Leptospira* spp. infection in different locations depends on environmental conditions such as climate and land terrain [26,28].

In the present study, males and females were found to be equally infected. Our findings agree with previous studies [29-31]. However, others [28,32] found that females were more affected than males. A few researchers [25,33] reported higher infection in males than females. An interesting finding on sex has been reported by Adesiyun *et al.* [34] in humans in the Caribbean nations that infection is higher in males. Our observation on *Leptospira* spp. infection in *R. norvegicus* in relation to age was not significant ( $p=0.26$ ) although figures for adult infection were apparently seen higher; adult rats (64.2%) compared to Juvenile (35.8%). In accordance with our results, other researchers found infection more in adults [24,25,30]. Benacer *et al.* [25] advocated aggressive habits of the adults, to be the reason for higher infection. With aggressive habit, adults are more prone to infection with *Leptospira* spp. Krøjgaard *et al.* [30] thought that higher infection in adults was a result of prolonged exposure to *Leptospira* spp. with age. Suepaul *et al.* [29] indicated that juveniles were at a higher risk compared to adults. No explanation for this result was given by the authors.

In the Caribbean nations, *Leptospira* spp. antibodies were detected in livestock, rodents, pets, wild species, and humans through ELISA, MAT, and other serological methods. The most common serovars found in animals, in Grenada, were *L. autumnalis*, *L. icterohaemorrhagiae*, and related serogroups [8,9]. In other Caribbean nations, most prevalent serovar in rats were *L. icterohaemorrhagiae*/

Copenhageni (in Barbados and Trinidad), *L. icterohaemorrhagiae*/Icterohaemorrhagiae (in Guadeloupe), and *L. icterohaemorrhagiae*/Copenhageni and *L. icterohaemorrhagiae*/Icterohaemorrhagiae (in Jamaica) [8]. Serovars were not identified that human cases were diagnosed in 14 Caribbean countries including Grenada. The dominant serovar in humans across the Caribbean was *L. interrogans* serovar Copenhageni [34,35]. Based on the prevalence of *Leptospira* spp. serovars in animals and rats, in the Caribbean, transmission of *Leptospira* spp. to humans from animals and rats is indicated.

## Conclusion

This is the first report of the prevalence of *Leptospira* serovars in Grenada based on PCR method. Our findings confirm active infection (9.3%) with *Leptospira* spp. in *R. norvegicus* in Grenada. Although the prevalence of active infection with *Leptospira* spp. in kidney of *R. norvegicus* from Grenada was not very high, *R. norvegicus* as reservoir should be considered as a high-risk factor for humans. Control campaign to reduce the population of rats in the country should be implemented. Further research to identify *Leptospira* spp. serovars in humans, rodents, animals, and pets should be taken up to prove the epidemiology of the disease in Grenada.

## Authors' Contributions

BS: Developed the concept, conducted PCR and interpreted the sequencing results, and assisted in manuscript writing. KT and NR: Trapping of the rats, anesthesia, collection of kidneys and helping in PCR. RS: Interpretation of results, and writing of the manuscript. All authors read and approved the final manuscript.

## Acknowledgments

We thank St George's University (Grenada, West Indies) for providing funding for this project under One Health Research Initiative (OHRI grants #06-14-10). The skilled technical assistance provided by Ms. Vanessa Matthew is greatly appreciated.

## Competing Interests

The authors declare that they have no competing interests.

## Publisher's Note

Veterinary World (Publisher of International Journal of One Health) remains neutral with regard to jurisdictional claims in published institutional affiliation.

## References

1. Ratet G, Veyrier FJ, Fanton d'Andon M, Kammerscheit X, Nicola MA, Picardeau M, *et al.* Live imaging of bioluminescent *Leptospira interrogans* in mice reveals renal colonization as a stealth escape from the blood defenses and antibiotics. *PLoS Negl Trop Dis* 2014;8:e3359.
2. Smythe LD, Wuthiekanun V, Chierakul W, Suputtamongkol Y, Tiengrim S, Dohnt MF, *et al.* The microscopic agglutination

- test (MAT) is an unreliable predictor of infecting *Leptospira* serovar in Thailand. *Am J Trop Med Hyg* 2009;81:695-7.
3. Palaniappan RU, Chang YF, Chang CF, Pan MJ, Yang CW, Harpending P, *et al.* Evaluation of lig-based conventional and real-time PCR for the detection of pathogenic *Leptospira*. *Mol Cell Probes* 2005;19:111-7.
  4. Riediger IN, Stoddard RA, Ribeiro GS, Nakatani SM, Moreira SDR, Skraba I, *et al.* Rapid, actionable diagnosis of urban epidemic leptospirosis using a pathogenic *Leptospira* lipL32-based real-time PCR assay. *PLoS Negl Trop Dis* 2017;11:e0005940.
  5. Tantibhedhyangkul W, Wongsawat E, Silpasakorn S, Waywa D, Saenyasiri N, Suesuay J, *et al.* Use of multiplex real-time PCR to diagnose scrub typhus. *J Clin Microbiol* 2017;55:1377-87.
  6. Pappas G, Papadimitriou P, Siozopoulou V, Christou L, Akritidis N. The globalization of leptospirosis: Worldwide incidence trends. *Int J Infect Dis* 2008;12:351-7.
  7. Everard CO, Fraser-Chanpong GM, James AC, Butcher LV. Serological studies on leptospirosis in livestock and chickens from Grenada and Trinidad. *Trans R Soc Trop Med Hyg* 1985;79:859-64.
  8. Peters A, Vokaty A, Portch R, Gebre Y. Leptospirosis in the Caribbean: A literature review. *Rev Panam Salud Publica* 2017;41:e166.
  9. Keenan J, Sharma R, Dicker R, Rayner J, Stone D. Seroprevalence of *Leptospira* in *Rattus norvegicus* in Grenada, West Indies. *West Indian Med J* 2009;58:114-7.
  10. Sharma RN, Thille K, Piechowski B, Tiwari K. Detection of serum antibodies against *Leptospira* spp. In brown rats (*Rattus norvegicus*) from Grenada, West Indies. *Vet World* 2019;12:696-9.
  11. Panti-May JA, Hernández-Betancourt SF, Torres-Castro MA, Machain-Williams C, Cigarroa-Toledo N, Sodá L, *et al.* Population characteristics of human-commensal rodents present in households from Mérida, Yucatán, México. *Manter J Parasit Divers* 2016; 5:1-6.
  12. Stoddard RA, Gee JE, Wilkins PP, McCausland K, Hoffmaster AR. Detection of pathogenic *Leptospira* spp. Through TaqMan polymerase chain reaction targeting the lipL32 gene. *Diagn Microbiol Infect Dis* 2009;64:247-55.
  13. Haake DA, Chao G, Zuerner RL, Barnett JK, Barnett D, Mazel M, *et al.* The leptospiral major outer membrane protein lipL32 is a lipoprotein expressed during mammalian infection. *Infect Immun* 2000;68:2276-85.
  14. Nally JE, Whitelegge JP, Bassilian S, Blanco DR, Lovett MA. Characterization of the outer membrane proteome of *Leptospira interrogans* expressed during acute lethal infection. *Infect Immun* 2007;75:766-73.
  15. Bedir O, Kilic A, Atabek E, Kuskucu AM, Turhan V, Basustaoglu AC, *et al.* Simultaneous detection and differentiation of pathogenic and nonpathogenic *Leptospira* spp. By multiplex real-time PCR (TaqMan) assay. *Pol J Microbiol* 2010;59:167-73.
  16. Woo TH, Smythe LD, Symonds ML, Norris MA, Dohnt MF, Patel BK, *et al.* Rapid distinction between *Leptonema* and *Leptospira* by PCR amplification of 16S-23S ribosomal DNA spacer. *FEMS Microbiol Lett* 1996;142:85-90.
  17. Saito M, Villanueva SY, Masuzawa T, Haraguchi Y, Ita S, Miyahara S, *et al.* The usefulness of semi-solid medium in the isolation of highly virulent *Leptospira* strains from wild rats in an urban area of Fukuoka, Japan. *Microbiol Immunol* 2015;59:322-30.
  18. Koizumi N, Miura K, Sanai Y, Takemura T, Ung TT, Le TT, *et al.* Molecular epidemiology of *Leptospira interrogans* in *Rattus norvegicus* in Hanoi, Vietnam. *Acta Trop* 2019;194:204-8.
  19. Backstedt BT, Buyuktanir O, Lindow J, Wunder EA Jr., Reis MG, Usmani-Brown S, *et al.* Efficient detection of pathogenic leptospires using 16S ribosomal RNA. *PLoS One* 2015;10:e0128913.
  20. Xue F, Yan J, Picardeau M. Evolution and pathogenesis of *Leptospira* spp.: Lessons learned from the genomes. *Microbes Infect* 2009;11:328-33.
  21. Latifah I, Abdul Halim A, Rahmat MS, Nadia MF, Ubil ZE, Asmah H, *et al.* Isolation by culture and PCR identification of lipL32 gene of pathogenic *Leptospira* spp. In wild rats of Kuala Lumpur. *Malays J Pathol* 2017;39:161-6.
  22. Fortes-Gabriel E, Carreira T, Vieira ML. First isolates of *Leptospira* spp. from rodents captured in Angola. *Am J Trop Med Hyg* 2016;94:955-8.
  23. Cosson JF, Picardeau M, Mielcarek M, Tatar C, Chaval Y, Suputtamongkol Y, *et al.* Epidemiology of *Leptospira* transmitted by rodents in Southeast Asia. *PLoS Negl Trop Dis* 2014;8:e2902.
  24. Loan HK, Van Cuong N, Takhampunya R, Kiet BT, Campbell J, Them LN, *et al.* How important are rats as vectors of leptospirosis in the Mekong delta of Vietnam? *Vector Borne Zoonotic Dis* 2015;15:56-64.
  25. Benacer D, Mohd Zain SN, Amran F, Galloway RL, Thong KL. Isolation and molecular characterization of *Leptospira interrogans* and *Leptospira borgpetersenii* isolates from the urban rat populations of Kuala Lumpur, Malaysia. *Am J Trop Med Hyg* 2013;88:704-9.
  26. Ivanova S, Herbretau V, Blasdell K, Chaval Y, Buchy P, Guillard B, *et al.* *Leptospira* and rodents in Cambodia: Environmental determinants of infection. *Am J Trop Med Hyg* 2012;86:1032-8.
  27. Yalin W, Lingbing Z, Hongliang Y, Jianmin X, Xiangyan Z, Xiaokui G, *et al.* High prevalence of pathogenic *Leptospira* in wild and domesticated animals in an endemic area of China. *Asian Pac J Trop Med* 2011;4:841-5.
  28. Pui CF, Bilung LM, Apun K, Su'ut L. Diversity of *Leptospira* spp. In rats and environment from urban areas of Sarawak, Malaysia. *J Trop Med* 2017;2017:3760674.
  29. Suepaul SM, Carrington CV, Campbell M, Borde G, Adesiyun AA. Seroepidemiology of leptospirosis in dogs and rats in Trinidad. *Trop Biomed* 2014;31:853-61.
  30. Krøjgaard LH, Villumsen S, Markussen MD, Jensen JS, Leirs H, Heiberg AC, *et al.* High prevalence of *Leptospira* spp. In sewer rats (*Rattus norvegicus*). *Epidemiol Infect* 2009;137:1586-92.
  31. Santos AA, Figueira CP, dos Reis MG, Costa F, Ristow P. Heterogenic colonization patterns by *Leptospira interrogans* in *Rattus norvegicus* from urban slums. *Braz J Microbiol* 2015;46:1161-4.
  32. Easterbrook JD, Kaplan JB, Vanasco NB, Reeves WK, Purcell RH, Kosoy MY, *et al.* A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *Epidemiol Infect* 2007;135:1192-9.
  33. Abdollahpour G, Shirzadi M, Darvish J, Mohammadi Z, Yazdani M, Mousazadeh A. Serological survey of leptospirosis in rodent of North Khorasan province, Northeast of Iran. *J North Khorasan Univ Med Sci* 2016;7:725-33.
  34. Adesiyun AA, Baboolal S, Suepaul S, Dookeran S, Stewart-Johnson A. Human leptospirosis in the Caribbean, 1997-2005: Characteristics and serotyping of clinical samples from 14 countries. *Rev Panam Salud Publica* 2011;29:350-7.
  35. Herrmann-Storck C, Saint-Louis M, Foucand T, Lamaury I, Deloumeaux J, Baranton G, *et al.* Severe leptospirosis in hospitalized patients, Guadeloupe. *Emerg Infect Dis* 2010;16:331-4.

\*\*\*\*\*