

# Isolation and Molecular Detection of *Leptospira* spp. from Rat Population in Selected Areas of Mymensingh District, Bangladesh

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**Abstract** *Leptospira*, the aetiology of leptospirosis, is one of the most important zoonotic bacteria that affects humans and animals all over the world. It has many carriers, but rat is considered as the first primary host for leptospirosis. Despite the fact that this disease is common in many countries, its presence in Bangladesh is little recognized. From a socioeconomic standpoint, it poses a serious risk to our cattle industry's sustainability. Therefore, the purpose of this work was to identify and characterize *Leptospira* from the rat population using molecular, dark field, and culture techniques, as well as to investigate its pathogenicity. Eighty rat samples were aseptically taken from both urban and rural parts of the Mymensingh district, including rat kidney and urine samples. The EMJH medium was used to first allow each sample to grow. Dark field microscopy was used to identify the bacterial isolates, and PCR was used for molecular detection. In urban areas, 16 out of 40 rat samples were positive (40%) whereas 10 out of 40 rat samples in rural regions were positive (25%). In both regions, it was discovered that adult rat samples had more *Leptospira* than juvenile rat samples, while female rat samples were more positive than male rat samples. We draw the conclusion that *Leptospira* is present in Bangladeshi rat populations in both urban and rural locations. The high pathogenicity of some serovars of this bacterium raises concerns about the hazards to public health posed by rat transmission of leptospirosis, even if the reported infection incidence is minimal.

**Keywords:** *Leptospira*, rat, isolation, molecular detection

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## 1. Introduction

Leptospirosis is one of the neglected zoonotic diseases in the world and is a global public health problem with high morbidity and mortality caused by the pathogenic bacteria of *Leptospira* [1,2,3]. *Leptospira* has 22 species, covering more than 300 serovars [4,5]. The pathogenic *Leptospira* can be infected in humans, rats, and dogs are most commonly caused by *Leptospira* serovar icterohaemorrhagiae and *Leptospira* serovar Hardjo was found in cattle [6]. In the United States of America, there are an estimated 873,000 infectious and 48,000 deaths reported each year due to Leptospirosis [7,8]. In South and Southeast Asia, the estimated annual incidence of leptospirosis is 17.97 and 55.54 per 100,000 populations [9].

Rats were known as *Leptospira*'s main reservoir hosts. They are also important since they can stay tainted for an extensive time without showing disease and transmitting pathogenic factors to humans and other animals [10].

Leptospirosis can cause fever in humans, and in severe cases, it can cause Weil's disease [11], which is caused by jaundice, acute renal failure, and bleeding [12] and bovine leptospirosis is the cause of mastitis [13], abortion [14], stillbirth or weak calves birth [15].

The infection is common in developing countries like India and Bangladesh due to tropical/subtropical climate, farming practices, humidity, and flooding, which are the major risk factors for the transmission of *Leptospira* [16]. In addition, it is expected that climate change will increase population growth, the expansion of urban areas, and the global burden of disease [17]. Leptospirosis alone will have a negative impact on rural communities in most Asian countries where the disease is endemic [18].

Very few studies have been conducted on leptospirosis in Bangladesh. *Leptospira* was detected serologically from the rural peoples of flood-prone districts. A high prevalence of low-level MAT reactivations was found in patients with jaundice high fever and healthy controls [19] and also from dengue-negative patients in Dhaka, Bangladesh [20] and cattle farms at Chittagong [21].

However, bacteriological identification of *Leptospira* field isolates from any host level has not yet been performed. There is no report on the prevalence and molecular characterization of *Leptospira* from the rat population in Bangladesh, as rats are the reservoir for *Leptospira*. Therefore, the present study has been designed for the first time in Bangladesh for the isolation, identification and molecular characterization of *Leptospira* spp. from rats in selected areas of the Mymensingh district. The purpose of this study was to investigate the prevalence of *Leptospira* and isolate and identify *Leptospira* spp. using cultural techniques and dark field microscopy in urban and rural rat populations. In addition, the Molecular characterization of these isolates was determined by PCR.

## 2. Materials and Methods

### 2.1. Ethical Approval

The Animal Welfare and Experimentation Ethics Committee of Bangladesh Agricultural University, Mymensingh, approved the methods described in this work [approval number AWEEC/BAU/2021(61)].

### 2.2. Sampling Areas

A cross-sectional study was conducted on rats collected from different areas (urban and rural) of Mymensingh district and transported to the Bacteriology Laboratory of Department of Microbiology and Hygiene, BAU (Bangladesh Agricultural University) for isolation, identification and molecular characterization of *Leptospira*. In this study, a total of 80 rats were selected from study areas (Table 1). Two samples (kidney and urine) were collected aseptically in the laboratory from each rat in Mymensingh district's selected urban and rural areas. For this, the total number of samples became 160 (Table 2).

Table 1. List of sample collecting areas

| Area                              | Location                 | Total rats | Male | Female | Adult | Juvenile |
|-----------------------------------|--------------------------|------------|------|--------|-------|----------|
| Urban areas                       | Kristapur                | 10         | 6    | 4      | 7     | 3        |
|                                   | Boubajar                 | 10         | 6    | 4      | 8     | 2        |
|                                   | Baghmara                 | 10         | 2    | 8      | 6     | 4        |
|                                   | Different sources at BAU | 10         | 2    | 8      | 5     | 5        |
| Rural areas                       | Sutiakhali               | 10         | 5    | 5      | 6     | 4        |
|                                   | Shambhuganj              | 10         | 3    | 7      | 7     | 3        |
|                                   | Chinar more              | 10         | 2    | 8      | 6     | 4        |
|                                   | Vangamari                | 10         | 3    | 7      | 7     | 3        |
| Total number of samples collected |                          | 80         | 29   | 51     | 52    | 28       |

BAU = Bangladesh Agricultural University

Table 2. Total number of samples collected from captured rats

| Location of Mymensingh areas | Number of rats collected | No. of kidney sample | No. of urine sample | Total No. of sample |
|------------------------------|--------------------------|----------------------|---------------------|---------------------|
| Urban area                   | 40                       | 40                   | 40                  | 80                  |
| Rural area                   | 40                       | 40                   | 40                  | 80                  |
| Total                        | 80                       | -                    | -                   | 160                 |

### 2.3. Capturing of Rats

We captured rats through traps equipped with metal cages and food baits such as dry fish, bread, peanut butter, soap, potato, biscuits, etc. Traps were placed in the evening, collected early in the morning the next day [22], and transferred alive to the laboratory for examination.

### 2.4. Host Identification and Collection of Sample

We killed captured rats using over dose anesthetic agent through the intra-peritoneal route [22]. Age and species, gender and life stage (juvenile/mature), each animal identification was carried out according to Medway [23] based on the phenotypic characteristics, such as fur color (ventral and dorsal), body weight, hindfoot and head-body length. The kidney samples were collected by postmortem and dissecting the dead rat body using a clean, sterile scalpel blade and scissors. Urine samples were collected by direct puncture of the urinary bladder of the rats using a sterile syringe (Figure 1).



Figure 1. Collection of sample

### 2.5. Processing of Sample

Kidney samples were processed by grinding with mortar and pestle, and kidney fluid was collected by squeezing for culturing [24]. The urine sample was ready to use for culture.

### 2.6. Isolation and Identification of *Leptospira*

Both samples were inoculated into modified Ellinghausen-McCullough Johnson-Harris (EMJH) medium (Becton, Dickinson Company) supplemented with 500 µg/ml or 312 µl/5ml of 5-fluorouracil (Nacalai Tesque Company) and incubated at 28°C-30°C until 7-28 days. *Leptospira* was identified by checking bacterial culture under dark field microscope at 40X and 100X. Colony characteristics such as thin helical structures with prominent hooked ends and motility developed on the culture medium after 7 days of incubation were recorded. Once *Leptospira* spp. was detected, sub-culturing was done [25] to obtain pure culture, which was kept as stock culture (growing culture with EMJH as it was and using 50% buffered glycerin) for preserving bacteria at - 20°C.

### 2.7. Detection and Confirmation of the *Leptospira* Isolates by PCR

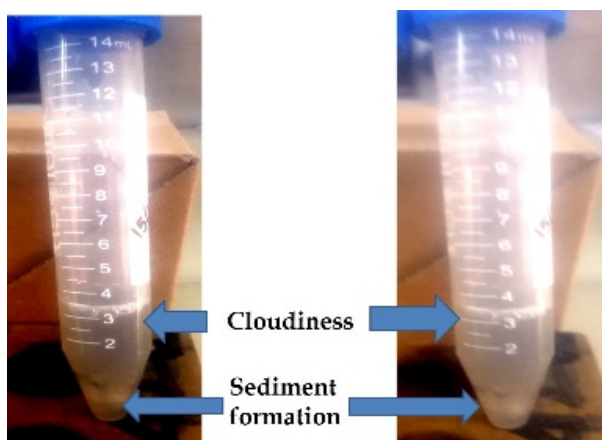
The DNA from both samples or culture samples was extracted for PCR using the Illustra™ Tissue and Cell Genomic Pre Mini Spin kit (GE Health care®), according to the manufacturer's instructions. PCR was performed using primers LA/LB [5'-GGC GGC GCG TCT TAA ACA TG-3'] and [5'-TTC CCC CCA TTG AGC AAG ATT-3'], which target the 16S rDNA gene for amplifying 331bp, were used to confirm the genus *Leptospira* [26]. The cycling conditions consisted of an initial denaturation at 94°C for 3 minutes, 35 cycles each of 94°C for 1 minute, 57°C for 1 minute, and 72°C for 2 minutes, and additional extension at 72°C for 10 minutes. The PCR products were analyzed by electrophoresis through a 1% TBE agarose gel (Promega, Madison, WI).

### 3. Results

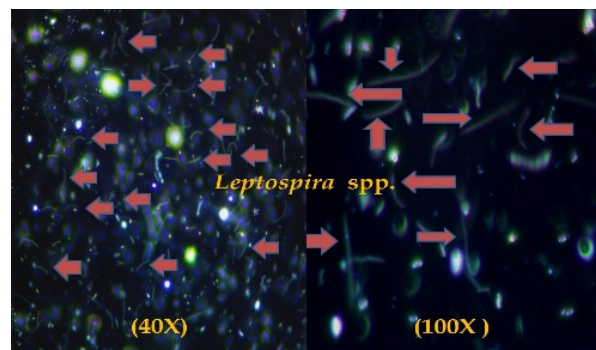
#### 3.1. Isolation and Identification of *Leptospira* Using Culture and Dark Field Microscopy

The active motility of *Leptospira* was observed, and the prevalence was estimated in selected urban and rural areas of the Mymensingh district according to the positivity in the culture medium. The formation of cloudiness with sediment indicated the growth of *Leptospira* on EMJH medium, and sometimes, an opalescent ring (Dinger zone) was also formed after growing bacteria (Figure 2). In culture media, dark field microscopy revealed thread-like helical structures with spiral-shaped or sometimes coiled or straight-shaped bacteria under 40X and 100X objectives (Figure 3).

In this study, out of 80 rat samples, 40 were collected from urban areas, 16 were found to be *Leptospira* positive, and 40 were collected from rural areas, 10 of which were *Leptospira* positive. Among 40 rat samples from urban areas, in 16 cases, both the kidney and urine samples were found to be *Leptospira* positive and out of 40 rat samples from rural areas, both the kidney and urine samples were *Leptospira* positive in 10 cases (Table 3).



**Figure 2.** Growth of *Leptospira* on EMJH medium (cloudiness and sediment formation)



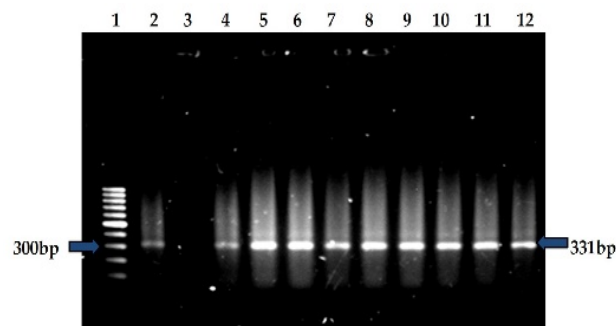
**Figure 3.** Thread-like thin helical structures with spiral-shaped or sometimes coiled or straight-shaped *Leptospira* spp. seen under dark field microscope

**Table 3.** Isolation and identification of *Leptospira* from rat samples

| Areas of Mymensingh areas | Number of rats collected | <i>Leptospira</i> positive (kidney sample) | <i>Leptospira</i> positive (urine sample) | Total <i>Leptospira</i> -positive Rat |
|---------------------------|--------------------------|--------------------------------------------|-------------------------------------------|---------------------------------------|
| Urban area                | 40                       | 16                                         | 16                                        | 16                                    |
| Rural area                | 40                       | 10                                         | 10                                        | 10                                    |
| Total                     | 80                       |                                            |                                           | 26                                    |

#### 3.2. Molecular Detection of *Leptospira* spp. by PCR

Out of total 40 samples from urban areas, 16 samples were PCR positive against 16S rDNA and 40 samples from rural areas, 10 samples were PCR positive against 16S rDNA (Figure 4).

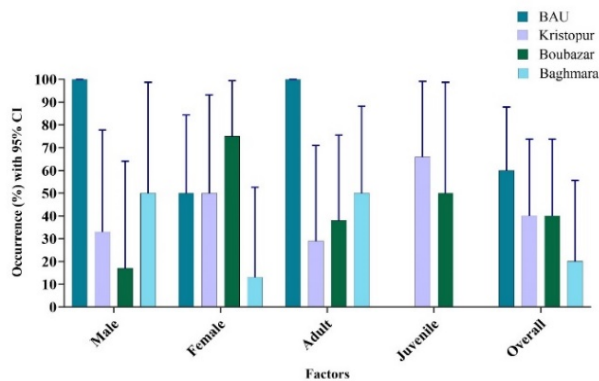


**Figure 4.** PCR assay to amplify 16S rDNA of *Leptospira* spp. isolates recovered from rat kidney and urine. Lane 1: 100bp size DNA marker; lane 2: Positive control; lane 3: Negative control without DNA and lane 4-12: Representative *Leptospira* spp. isolates

#### 3.3. Occurrence of *Leptospira* in Different Urban Areas

In the present study, the highest occurrence of leptospira was 60% (95% CI: 87.84% - 26.24%) from the different sources at BAU, followed by 40% (95% CI: 73.76% - 12.16%) from both Kristopur and Boubajar and 20% (95% CI: 56.61% - 2.52%) from Baghmara. Based on sex, the highest occurrence of *Leptospira* from male rat was 100% (95% CI: 100% - 34.20%) from different

sources at BAU, followed by 50% (95% CI: 98.74% - 1.26%), 33% (95% CI: 77.72% - 4.33%), and 17% (95% CI: 64.12% - 0.42%) from Baghmara, Kristopur, and Boubajar, respectively. In contrast, the highest occurrence of *Leptospira* from female rat was 75% (95% CI: 99.37% - 19.41%) from Boubajar, 50% (95% CI: 93.24% - 6.76%) from Kristopur, 50% (95% CI: 84.30% - 15.70%) in different sources at BAU and 13% (95% CI: 52.65% - 0.32%) from Baghmara. In case of age, the highest occurrence of *Leptospira* from adult rat was 100% (95% CI: 100% - 47.82%) from different sources at BAU followed by 50% (95% CI: 88.19% - 11.81%), 38% (95% CI: 75.51% - 8.52%), 29% (95% CI: 70.96% - 3.67%) from Baghmara, Boubajar and Kristopur. On the contrary, the highest occurrence of *Leptospira* from juvenile rat was 67% (95% CI: 99.16% - 9.43%) from Kristopur, followed by 50% (95% CI: 98.74% - 1.26%) from Boubajar, and no samples showed positive at Baghmara and different sources at BAU (Figure 5). Overall occurrence of *Leptospira* in urban areas was 38%, 42%, 50%, and 21% in male, female, adult, and juvenile rats, respectively.

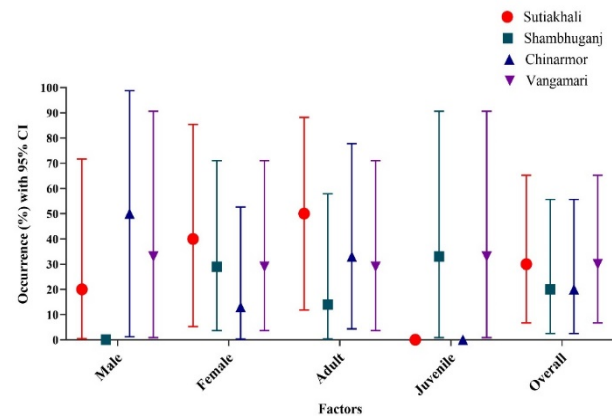


**Figure 5.** Occurrence of *Leptospira* in different urban areas rat according to location, sex, and age

### 3.4. Occurrence of *Leptospira* in Different Rural Areas

In this study, the highest occurrence of *leptospira* in rats in different rural areas was 30% (95% CI: 65.25% - 6.67%) in both Sutiakhali and Vangamari, followed by 20% (95% CI: 55.61% - 2.52%) in both Shambhuganj and Chinarmor. In case of sex, the highest occurrence of *Leptospira* from male rat was 50% (95% CI: 98.74% - 1.26%) from Chinarmor followed by 33% (95% CI: 90.57% - 0.84%), 20% (95% CI: 71.64% - 0.51%) from Vangamari, Sutiakhali, respectively and no samples showed positive result at Shambhuganj. In contrast, the highest occurrence of *Leptospira* in female rats was 40% (95% CI: 85.34% - 5.27%) from Sutiakhali followed by 29% (95% CI: 70.96% - 3.67%) from both Shambhuganj and Vangamari and 13% (95% CI: 52.65% - 0.32%) from Chinarmor. In case of age, the highest occurrence of *Leptospira* from adult rats was 50% (95% CI: 88.19% - 11.81%) from Sutiakhali followed by 33% (95% CI: 77.72% - 4.33%), 29% (95% CI: 70.96% - 3.67%), 14% (95% CI: 57.87% - 0.36%) from Chinarmor, Vangamari, and Shambhuganj, respectively. On the contrary, the highest occurrence of *Leptospira* from juvenile rats was 33% (95% CI: 90.57% - 0.84%) from Shambhuganj and Vangamari followed by

no samples showed positive at both Sutiakhali and Chinarmor (Figure 6). Overall occurrence of *Leptospira* in rural areas was 23%, 26%, 35%, and 14% in male, female, adult, and juvenile rats, respectively.



**Figure 6.** Occurrence of *Leptospira* in different rural areas rat according to location, sex, and age

## 4. Discussion

In order to reveal whether rats in Bangladesh carry *Leptospira*, we performed isolation, identification and molecular characterization of *Leptospira* bacteria from the kidney and urine of rats captured in selected urban and rural areas of Mymensingh district causing *Leptospirosis* disease in Bangladesh.

We detected *Leptospira* spp. from rat populations in selected urban and rural areas by using EMJH culture medium, dark field microscopy and polymerase chain reaction (PCR) and the overall prevalence was 33% of which 40% from urban areas and 25% from rural areas. These results of isolation are related to the findings of some previous studies [26,27,28]. Moreover, Fonseca *et al.* reported that PCR is the main tool for the successful early detection of genus and pathogenic species of *Leptospira* [29]. Tulsiani *et al.* asserted in their study that the results of sensitivity and specificity analyses for the various diagnostic tests showed that isolation by culture, the gold standard for diagnosing leptospiral shedding, had low specificity (40%) but perfect (100%) sensitivity when compared to the PCR results [30].

In the present research, we found that the occurrence of *Leptospira* in the urban rat population (40%) is more than in the rural rat population (25%). We showed some reasons for this differentiation of the occurrence based on our selected areas. Regarding urban locations, we caught rats in residential areas, crowded places, poorly managed trash, wet markets (which offer a wide variety of raw foods), food storage rooms, and jute bags that create temporary foraging grounds for rats. These regions also draw rodents, stray dogs, and food sources. In case of rural areas, rats were trapped from hawker centres, restaurants and roadside stalls selling cooked food, rice storage areas of households and rice fields. One of the most common strains, according to Vedhagiri *et al.* [31], was *L. borgpetersenii* serovar Javanica, which was also isolated from rice fields and rats that were captured in rural Tamil Nadu, India. This serovar existed among the

urban rats that could be described by the migration of infected rats with the transportation of goods, rice and animals from the rural areas to the urban areas. If we associate our findings with their findings, this is the probable reason that can be explained the high number of positive rats infected with *Leptospira* spp., where raw foods had been brought from rural areas to be sold in the urban areas.

In our study, we found that the occurrence of *Leptospira* in female rats was more than the male rats in both areas. This occurrence is different from the results found by Benacer et al. [26]; Lekhal et al. [32] and similar to the results found by Easterbrook et al. [33]; Desvars-Larrive et al. [34]. Agudelo-Florez et al. [35] reported that there was no correlation between sex, age, and health condition of the host and prevalence of the infection.

In this study we found that the occurrence of *Leptospira* positive is more in adult rats than juvenile rats, which is similar to the findings of Benacer et al. [26]; Santos et al. [36]. Mohamed et al. [37] pointed out that the adult rats who were aggressive, fighting, and biting, may have facilitated the transmission of *Leptospira*. In contrast, young rats cannot move like adult rats and they experience less exposure to the pathogen that's why young rats are less *Leptospira* positive than adult rats.

The limited sample size ( $n = 80$ ) and number of locations examined ( $n = 8$ ) are the main limitations of our study. In order to provide a more comprehensive picture of the epidemiology of leptospirosis in urban and rural rats, a larger sample size would have improved the statistical analysis's power.

## 5. Conclusion

This study provides critical insights into the prevalence of *Leptospira* spp. in rat populations from both urban and rural areas of Mymensingh district, Bangladesh. The overall prevalence of *Leptospira* was 32.5%, with urban areas showing a higher prevalence (40%) compared to rural areas (25%). Female rats and adult rats were more frequently found to carry *Leptospira* spp. than their male and juvenile counterparts. These findings underscore the role of rats as significant reservoirs for *Leptospira* spp., presenting a potential public health risk for leptospirosis transmission to humans and animals in Bangladesh. Despite the relatively low infection rates, the presence of highly pathogenic serovars raises concerns about the zoonotic threat and its socio-economic impact, particularly on the livestock industry. The study highlights the need for increased awareness, enhanced diagnostic capabilities, and preventive measures to mitigate the risk of leptospirosis outbreaks. Future research should focus on a broader geographical scope, serovar-level identification, and evaluation of environmental and seasonal factors influencing *Leptospira* spp. transmission in Bangladesh.

## Conflict of Interest

The authors assert that they do not possess any conflicts of interest.

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## References

- [1] Adler, B., "Pathogenesis of leptospirosis: Cellular and molecular aspects", *Veterinary Microbiology*, 172, 353–358. 2014.
- [2] Zuermer, R.L., "Host response to leptospira infection", *Current Topics in Microbiology and Immunology*, 387, 223–250. 2015.
- [3] Karpagam, K.B., Ganesh, B., "Leptospirosis: a neglected tropical zoonotic infection of public health importance—an updated review", *European Journal of Clinical Microbiology & Infectious Diseases*, 39, 835–846. 2020.
- [4] Abela-Ridder, B., Sikkema, R., Hartskeerl, R.A., "Estimating the burden of human leptospirosis", *International Journal of Antimicrobial Agents*, 36, S5–S7. 2010.
- [5] Cerqueira, G.M., Picardeau, M., "A century of *Leptospira* strain typing", *Infection, Genetics and Evolution*, 9, 760–768. 2009.
- [6] Jumper, W.L., "Infectious Agents", *Bovine Reproduction*, 733–741. 2021.
- [7] Ko, A.I., Goarant, C., Picardeau, M., "Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen", *Nature Reviews Microbiology*, 710, 736–747. 2009.
- [8] Bandara, M., Ananda, M., Wickramage, K., Berger, E., Agampodi, S., "Globalization of leptospirosis through travel and migration", *Global Health*, 10, 1–9. 2014.
- [9] Costa, F., Hagan, J.E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M.S., Stein, C., Abela-Ridder, B., Ko, A.I., "Global Morbidity and Mortality of Leptospirosis: A Systematic Review", *PLOS Neglected Tropical Diseases*, 9, e0003898. 2015.
- [10] Nakamura, I., Hang'ombe, B.M., Sawa, H., Kobayashi, S., Orba, Y., Ishii, A., Thomas, Y., Isozumi, R., Yoshimatsu, K., Mweene, A.S., Takada, A., Sugimoto, C., Arikawa, J., "Cross-Reactivity of Secondary Antibodies against African Rodents and Application for Sero-Surveillance", *Journal of Veterinary Medical Science*, 75, 819–825. 2013.
- [11] Faine, P.P., Adler, S., Bolin, B., *Leptospira and leptospirosis*, Melbourne, Australia, MedScience.1999.
- [12] McBride, A.J.A., Athanazio, D.A., Reis, M.G., Ko, A.I., "Leptospirosis", *Current Opinion in Infectious Diseases*, 18, 376–386. 2005.
- [13] Garoussi, M.T., Atarie, S., Vodjgani, M., Gharagozloo, F., Vand e ussefi, J., "The prevalence and control of bovine mastitis in *Leptospira* outbreak", *Comparative Clinical Pathology*, 26, 189–192. 2017.
- [14] Grégoire, F., Bakinahe, R., Petitjean, T., Boarbi, S., Delooz, L., Fretin, D., Saulmont, M., Mori, M., "Laboratory Diagnosis of Bovine Abortions Caused by Non-Maintenance Pathogenic *Leptospira* spp.: Necropsy, Serology and Molecular Study Out of a Belgian Experience", *Pathogens*, 9, 413. 2020.
- [15] Loureiro, A.P., Lilienbaum, W., "Genital bovine leptospirosis: A new look for an old disease", *Theriogenology*, 141, 41–47. 2020.
- [16] Bharti, A.R., Nally, J.E., Ricaldi, J.N., Matthias, M.A., Diaz, M.M., Lovett, M.A., Levett, P.N., Gilman, R.H., Willig, M.R., Gotuzzo, E., Vinetz, J.M., "Leptospirosis: A zoonotic disease of global importance", *The Lancet Infectious Diseases*, 757–771. 2003.
- [17] Hochrainer, S., Mechler, R., "Natural disaster risk in Asian megacities: A case for risk pooling?", *Cities*, 28, 53–61. 2011.
- [18] Narkkul, U., Thaipadungpanit, J., Srisawat, N., Rudge, J.W., Thongdee, M., Pawarana, R., Pan-ngum, W., "Human, animal, water source interactions and leptospirosis in Thailand", *Scientific Reports*, 11, 1–13. 2021.
- [19] Morshed, M.G., Konishi, H., Terada, Y., Arimitsu, Y., Nakazawa, T., "Seroprevalence of leptospirosis in a rural flood prone district of Bangladesh", *Epidemiology & Infection*, 112, 527–531. 1994.

- [20] LaRocque, R.C., Breiman, R.F., Ari, M.D., Morey, R.E., Janan, F.A., Hayes, J.M., Hossain, M.A., Brooks, W.A., Levett, P.N., "Leptospirosis during Dengue Outbreak, Bangladesh", *Emerging Infectious Diseases*, 11, 766. 2005.
- [21] Parvez, M.A., Prodhan, M.A.M., Rahman, M.A., Faruque, M.R., "Seroprevalence and Associated Risk Factors of Leptospira interrogans Serovar Hardjo in Dairy Cattle of Chittagong, Bangladesh", *Pakistan Veterinary Journal*, 35, 350–354. 2015.
- [22] Nelson, L., Clark, F.W., "Correction for Sprung Traps in Catch/Effort Calculations of Trapping Results", *Journal of Mammalogy*, 54, 295–298. 1973.
- [23] Medway, L.G.G.H., "The wild mammals of Malaya (Peninsular Malaysia) and Singapore" - 2. ed., 1978.
- [24] Passos, E.D.C., Vasconcellos, S.A., Ito, F.H., Yasuda, P.H., Junior, R.N., "Isolamento de leptospiros a partir do tecido renal de hamsters experimentalmente infectados com Leptospira interrogans sorotipo pomona. Emprego das técnicas ... com 5-fluor-uracil ou o sulfato de neomicina", *Revista de la Facultad de Medicina Veterinaria y de Zootecnia*, 25, 221–235. 1988.
- [25] Ellis, W.A., McParland, P.J., Bryson, D.G., Thiermann, A.B., Montgomery, J., "Isolation of leptospires from the genital tract and kidneys of aborted sows", *Veterinary Record*, 118, 294–295. 1986.
- [26] Benacer, D., Zain, S.N.M., Amran, F., Galloway, R.L., Thong, K.L., "Isolation and molecular characterization of Leptospira interrogans and Leptospira borgpetersenii Isolates from the urban rat populations of Kuala Lumpur, Malaysia", *American Journal of Tropical Medicine and Hygiene*, 88, 704–709. 2013.
- [27] Pellizzaro, M., Martins, C.M., Yamakawa, A.C., daCunha Ferraz, D., Morikawa, V.M., Ferreira, F., dos Santos, A.P., Biondo, A.W., Langoni, H., "Molecular detection of Leptospira spp. in rats as early spatial predictor for human disease in an endemic urban area", *PLoS One*, 14, e0216830. 2019.
- [28] Latifah, I., Abdul Halim, A., Rahmat, M., Nadia, M.F., Ubil, Z.E., Asmah, H., Akmar, I.S., Picardeau, M., Haslina, O.S., Nasir, M.A., "Isolation by culture and PCR identification of LipL32 gene of pathogenic Leptospira spp. in wild rats of Kuala Lumpur, Malays", *The Journal of Pathology*, 39, 161–166. 2017.
- [29] Fonseca, C.D.A., Teixeira, M.M.G., Romero, E.C., Tengan, F.M., da Silva, M.V., Shikanai-Yasuda, M.A., "Leptospira DNA detection for the diagnosis of human leptospirosis", *Journal of Infection*, 52, 15–22. 2006.
- [30] Tulsiani, S.M., Graham, G.C., Dohnt, M.F., Burns, M.A., Craig, S.B., "Maximizing the chances of detecting pathogenic leptospires in mammals: the evaluation of field samples and a multi - sample - per - mammal, multi - test approach", *Annals of Tropical Medicine & Parasitology*, 105, 145–162. 2011.
- [31] Vedhagiri, K., Natarajaseenivasan, K., Prabhakaran, S.G., Selvin, J., Narayanan, R., Shouche, Y.S., Vijayachari, P., Ratnam, S., "Characterization of Leptospira borgpetersenii isolates from field rats (Rattus norvegicus) by 16S rRNA and lipL32 gene sequencing", *Brazilian Journal of Microbiology*, 41, 150–157. 2010.
- [32] Lekhal, L., Harran, E., Aragon, A., Groud, K., Le Guyader, M., Kaidi, R., Khelef, D., Djelouadi, Z., "First Molecular Detection of Pathogens Leptospira in Common Rodent Captured in North Algeria Urban Areas", *Tropical Medicine and Infectious Disease*, 7, 335. 2022.
- [33] Easterbrook, J.D., Kaplan, J.B., Vanasco, N.B., Reeves, W.K., Purcell, R.H., Kosoy, M.Y., Glass, G.E., Watson, J., Klein, S.L., "A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA", *Epidemiology & Infection*, 135, 1192–1199. 2007.
- [34] Desvars-Larrive, A., Smith, S., Munimanda, G., Bourhy, P., Waigner, T., Odom, M., Gliga, D.S., Walzer, C., "Prevalence and risk factors of Leptospira infection in urban brown rats (Rattus norvegicus), Vienna, Austria", *Urban Ecosystems*, 23, 775–784. 2020.
- [35] Agudelo-Flórez, P., Londoño, A.F., Quiroz, V.H., Ángel, J.C., Moreno, N., Loaiza, E.T., Muñoz, L.F., Rodas, J.D., "Prevalence of Leptospira spp. in urban rodents from a groceries trade center of Medellín, Colombia", *American Journal of Tropical Medicine and Hygiene*, 81, 906–910. 2009.
- [36] Santos, A.A.N., dos Santos Ribeiro, P., da França, G.V., Souza, F.N., Ramos, E.A.G., Figueira, C.P., Reis, M.G., Costa, F., Ristow, P., "Leptospira interrogans biofilm formation in Rattus norvegicus (Norway rats) natural reservoirs", *PLOS Neglected Tropical Diseases*, 15, e0009736. 2021.
- [37] Mohamed-Hassan, S. N., Bahaman, A. R., Mutalib, A. R., Khairani-Bejo, S., "Prevalence of Pathogenic Leptospires in Rats from Selected Locations in Peninsular Malaysia", *Research Journal of Animal Science*, 6, 12–25. 2012.

