Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 26 No. 2 Ser. No. 91 2025

# IRANIAN JOURNAL OF VETERINARY RESEARCH

**Original Article**

# Seroprevalence and carrier status of *Leptospira* spp. in rats captured in the central northern region of Algeria

**Lekhal, L.[1*]; Amara Korba, A.[2] and Ghalmi, F.[3]**

[1]Laboratory of Biotechnologies related to Animal Reproduction, Institute of Veterinary Sciences, route de Soumaa, Blida 09000, Algeria, and Department of Veterinary Sciences, Institute of Veterinary Sciences, Saad Dahlab University Blid1, Blida 09000, Algeria; [2]Leptospira Unit, Pasteur Institute Algeria, Rue 1 Docteur Laveran, El Hamma Anassers Algiers, Algeria; [3]Dapertment of Veterinary Sciences, Higher National Veterinary School, Oued Smar, Algiers 016000, Algeria, and Local Animal Resources Management Laboratory, Higher National Veterinary School, Oued Smar, Algiers 016000, Algeria

[*]***Correspondance:*** *L. Lekhal, Laboratory of Biotechnologies related to Animal Reproduction, Institute of Veterinary Sciences, route de Soumaa, Blida 09000, Algeria, and Department of Veterinary Sciences, Institute of Veterinary Sciences, Saad Dahlab University Blid1, Blida 09000, Algeria. E-mail: lilalekhal.vet09@yahoo.com*

10.22099/ijvr.2025.51165.7583

(Received 8 Sept 2024; revised version 27 Mar 2025; accepted 21 Apr 2025)



## Abstract

**Background:** Leptospirosis is a zoonosis with a large distribution in the globe, and *Leptospira* spp. is responsible for the disease. Mammalians can serve as reservoir hosts of the bacteria; however, rodents, particularly rats, are known to be the most important reservoir, principally, for *Leptospira interrogans* serovar Icterohaemorrhagiae. In Algeria, few data are available concerning the circulation of the *Leptospira* bacterium in human and in animals including rodents. **Aims:** Our study aimed to bring to light the importance of rats as reservoir host of *Leptospira* spp. in the city of Blida, Algeria. **Methods:** A total of 100 rats including 88 *Rattus norvegicus* and 12 *Rattus rattus* were captured, their serums were tested for antibodies by Microscopique Agglutination Test (MAT), and their kidneys and livers were subjected to culture in Ellinghausen, McCullough, Johnson and Harris (EMJH) medium. **Results:** Our study revealed a sero-prevalence of 43% (95% CI: 33.3-52.7%), the most common infecting serogroup was Icterohaemorrhagiae 11% (11/100), and the highest titer was register for the serogroup Canicola 1:2560. No statistical difference was recorded between the two sexes, the classes of age, and the rat's species; However, rats captured in urban area seemed to be more infected than those captured in rural area. Organs culture confirmed the carrying status of the bacteria with prevalence of 8%. **Conclusion:** Our survey confirms the role of rat as reservoir host of *Leptospira*, and provides valuable data on the epidemiology of leptospirosis in this animal. Therefore, rat population control in the city of Blida is important to prevent outbreaks of leptospirosis in human and in other animals.

**Key words:** Blida, Culture, Leptospirosis, MAT, Rat

## Introduction

Leptospirosis, a zoonosis with worldwide distribution and *Leptospira* pathogenic genus, is the causative agent of the disease (Tilley *et al*., 2004). Annually, leptospirosis affects over than one million people with 60-000 death (Costa *et al*., 2015). Leptospires are divided into more than 250 pathogenic serovars grouped into at least 32 serogroups (Caimi and Ruybal, 2020) the contamination can occur through contact with infected urine or contaminated water. The bacteria penetrates the body through aerosols inhalation, conjunctiva and mucous membranes and even through skin lacerations (Musso and La Scola, 2013).

Numerous of wild and domestic animals can serve as reservoir host of the bacteria (Bharti *et al*., 2003); leptospires colonize their kidneys and are excreted into the environment through their urine constituting an indirect way of contamination (Samrot *et al*., 2021).

Mostly, rodents are recognized as the major reservoirs of *Leptospira* spp (Garcia-Lopez *et al*., 2024) with a seroprevalence ranging from 18.3% to 96% among urban rodents across the world (Noh *et al*., 2024) and rats as particular hosts for the Icterohaemorrhagiae serogroup (Garcia-Lopez *et al*., 2024).

In Algeria, the most predominant circulating *Leptospira* species in human leptospirosis cases is *interrogans*; serovar Icterohemorrhagiae and serovar Canicola (Afiri, 2013; Afiri *et al*., 2013). *Leptospira interrogans* species was also associated to stray dogs (Zaidi *et al*., 2018), and to cattle; serovar Hardjo (Derdour *et al*., 2017; Benseghir *et al*., 2020).

Microscopic Agglutination Test (MAT) is considered as the serological standard test for the diagnosis of leptospirosis (Ahmad *et al*., 2005). However, the detection of leptospires by culture is considered as the definitive diagnosis test of the disease (Adler and de laPeña Moctezuma, 2010).

Knowledge of the epidemiology of the infection in reservoirs is important to establish an effective prophylaxis program, and to evaluate the risk of transmission to other animals and to human. The present study aimed to determine the seroprevalence of *Leptospira* in rats captured in the city of Blida and identify the circulating serovars. Also, we aimed to isolate the bacteria by culturing to confirm the carriage status of rats.

## Materials and Methods

The laboratory tests were performed at the Institute Pasteur Algeria.

The study was conducted under the authorization number 178/ISVPG/14, established by the Institute of Veterinary Science; University Bida1, Algeria and agreed by the Institute Pasteur Algeria.

### Study area and trapping of rats

Alive rodents were captured in the city of Blida during a routine rodent's population control of the Service d'Hygiène of Blida, under the authorization number SHC/123/2014. Rodents were caught from September 2014 to May 2015 using spring cages of approximately [30 cm, 15 cm, and 15 cm] size. Trap locations were selected according to some criteria; such as confirmed existence of rodents, security and help. In total rodents were captured in five urban areas "36.468197, 2.821059", "36.471856, 2.829977", "36.500609, 2.853508", "36.481361, 2.855541", "36.465242, 2.836651" and in three rural habitats "36.553899, 2.795626", "36.498020, 2.754173", "36.455496, 2.813976" (Fig. 1). Briefly, five to ten cages were placed by site and every morning the traps were collected. Rat species were identified according to their morphological characteristics such as the head and body length, tail length, ear and hind foot length (Ahmim, 2004). Sex and age were determined by external examination (Herbreteau *et al*., 2011).

### Samples collection

Rats were anesthetized and euthanized according to the protocol admitted by the Institute Pasteur Algeria, and the "Laboratoire des biotechnologies liées à la reproduction animale" (LBRA); Institute of Veterinary Science, University of Blida1.

Rats were bled from the heart and 2 to 5 ml of blood was collected depending on the size of the rodent. Kidneys and livers were aseptically removed and placed in sterilized sample bottles and were transported immediately under cold to the Institute Pasteur Algeria.

### Microscopic agglutination test

Blood samples were centrifuged and the obtained serum was stored at -20°C until processing by MAT.

MAT is thought to be the reference test for the diagnosis of leptospirosis and is specific to the serogroup (Levett, 2001). In our study, MAT was performed following the protocol established by the Institute Pasteur Algeria (Postic *et al*., 2000).

Living leptospires obtained from CNRLIPP-France were sub-cultured in EMJH medium, and were then used as antigens at 4 to 10 days of growth. The strain used corresponded to 23 pathogenic serovars and one saprophytic serovar (Table 1). Patoc as saprophytic serovar was used to indicate nonspecific reactions to others serovar not included in the panel (Loan *et al*., 2015). The serovar reacting with the highest titer was considered as the infecting serovar (O'Keefe *et al*., 2002; Geisen *et al*., 2007).

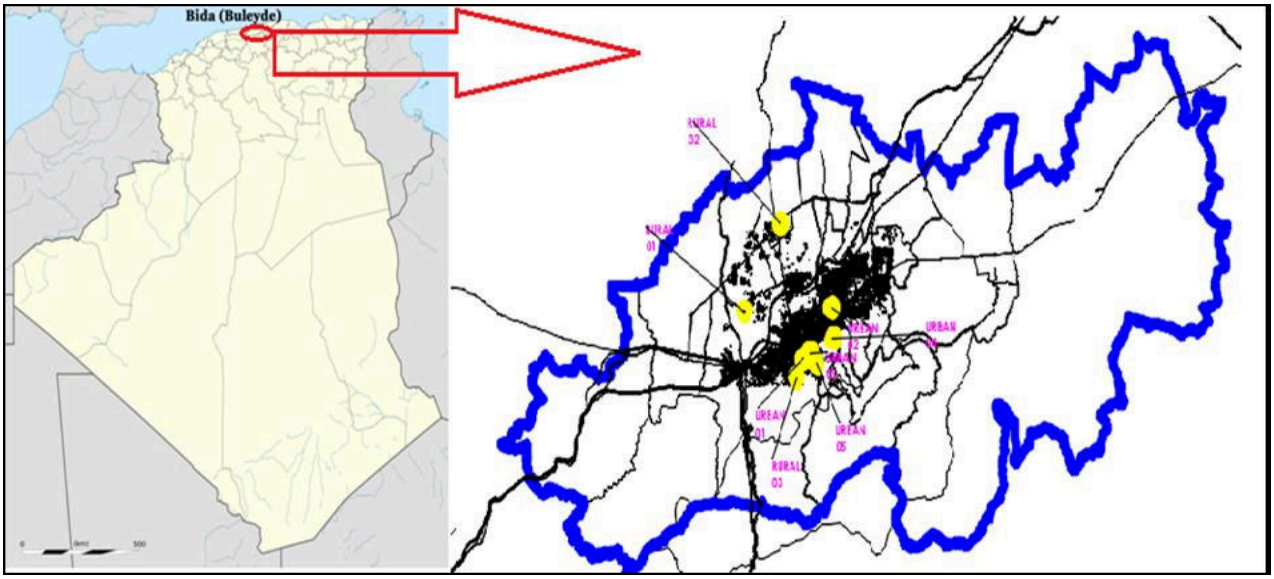


**Fig. 1:** Geographical location of the city of Blida, Algeria, and the sites of capture

**Table 1:** Panel of *Leptospira* strains used for MAT

| Serogroups | Serovars | Abbreviation |
|---|---|---|
| Australis | Australis | Aust |
| Autumnalis | Autumnalis | Autu |
| Bataviae | Bataviae | Bat |
| Canicola | Canicola | Can |
| Ballum | Castellonis | Cast |
| Cynopteri | Cynopteri | Cyno |
| Grippotyphosa | Grippotyphosa | Grip |
| Hebdomadis | Hebdomadis | Heb |
| Sejroe | Sejroe | Sej |
| | Hardjo | Hardj |
| Icterohaemorrhagiae | Icterohaemorrhagiae | Ict |
| | Icterohaemorrhagiae verdun | Ihv |
| Panama | Panama | Pan |
| Louisiana | Louisiana | Loui |
| Pomona | Pomona | Pom |
| Pyrogenes | Pyrogenes | Pyr |
| Tarassovi | Tarassovi | Tar |
| Celledoni | Celledoni | Cell |
| Djasiman | Djasiman | Dja |
| Mini | Mini | Mini |
| Sarmin | Sarmin | Sar |
| Shermani | Shermani | Sher |
| Javanica | Javanica | Jav |
| Semaranga | Patoc | Pat |

Rat serum was considered as positive when a titer of 1:20 or higher was obtained with at least one serovar (Vanasco *et al*., 2001). Antigens were first added to serum specimens to detect the positives one at dilution 1:20. Positive serums were then diluted in serial (1:20, 1:40, 1: 80, … 1:5120) and the corresponding antigen was added to determine the end titer.

The mixture of serum-antigen was observed under dark-field microscope after the incubation of 1 h at 37°C and a titer was defined as the highest dilution giving agglutination of 50% of free living leptosires used as antigen comparing to that of the negative control (Postic *et al*., 2000).

### *Leptospira* culture

Culture was performed using Ellinghausen McCullough Johnson Harris (EMJH) medium. Renal and liver tissues were crushed with sterile blades and transferred aseptically to tubes containing the medium, decimal dilution tubes were then incubated at 28-30°C and were examined weekly using a dark-field microscope for 8 weeks (Postic *et al*., 2000). The contamination was controlled by passing the medium throw filter of 0.45 µm and 0.22 µm. Finally, Positive cultures were purified and sub-cultured in EMJH medium.

### Statistical analysis

To determine if there were any statistically significant relationships between the sero-positivity and other factors such as sex, age, rat's species and different trap areas, Chi-square analysis was used with a confidence interval of 95%. Difference was considered significant when $P<0.05$. The data were analyzed using Excel® 2007.

Confidence interval of 95% was calculated according to the formula (Thierry, 2012):

$$IC = Z\alpha \pm \sqrt{PQ/N}$$

Where,
P: The observed frequency
Q= 1-P
$Z\alpha = 1.96$
N: The sample size

## Results

### Microscopic agglutination test

In total, antibodies were detected in 43 of the 100 rat serum samples (43%, 95% CI: 33.3%-52.7%), among which 20 sera reacted to only one serovar, 18 sera reacted to multiple serovar with an infecting serovar as the one giving the highest titer (O'Keefe *et al*., 2002; Geisen *et al*., 2007) and 5 sera reacted to multiple serovar with no dominant serovar. In total, 94 reactions were recorded by MAT (Fig. 2).

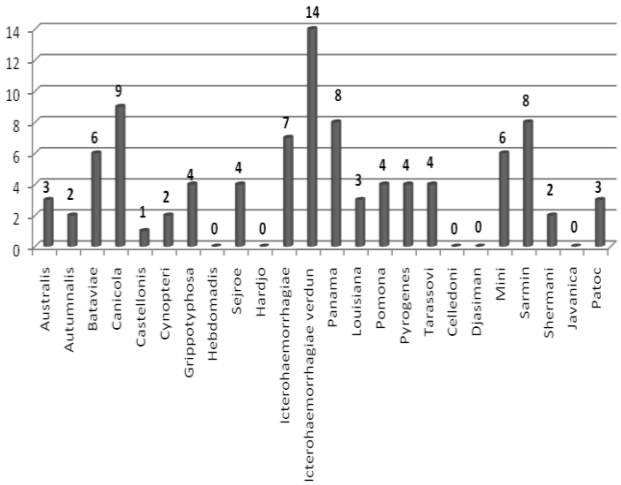


**Fig. 2:** Frequency of infection of samples positive to each serovar tested. The number of samples positive to each serovar adds up to more than the actual total number of samples tested because many samples were positive to more than one serovar. Number of reactions by MAT to each serovar (total reactions 94)

The proportions of the predominant infecting serogroups were: Icterohaemorrhagiae (11%, 11/100), Canicola (6%, 6/100), Grippotyphosa and Australis (3%, 3/100) (Table 2). The highest antibody titer was register for serovar Canicola 1:2560. However, for other serovars, the titer comprised among 20 to 640 (Table 3). Concerning the 23 sera reacting to multiple serovars 74 reactions were recorded (Table 4).

Of 100 captured rats, twenty two ($22^{+}/53$) females, twenty one ($21^{+}/47$) males, thirty two ($32^{+}/73$) adults, eleven (11/27) subadults, six ($6^{+}/12$) *Rattus rattus* and thirty seven ($37^{+}/88$) *Rattus norvegicus* were positive by MAT (Table 5).

According to the Chi-square analysis, there was no statistical association between MAT sero-positive rates and sex (P=0.75), age of rodents (P=0.78), and rodent's species (P=0.6) (Table 5).

**Table 2:** Number of significant reactions to the infecting serovar (number of rats with determined infecting serovar 38)

| Serogroup | Serovar | Infecting serovar (38 rats) |
|---|---|---|
| Icterohaemorrhagiae | Icterohaemorrhagiae verdun | 9 |
| | Icterohaemorrhagiae | 2 |
| Canicola | Canicola | 6 |
| Grippotyphosa | Grippotyphosa | 3 |
| Australis | Australis | 3 |
| Panama | Panama | 2 |
| Sarmin | Sarmin | 2 |
| Louisiana | Louisiana | 2 |
| Mini | Mini | 2 |
| Automnalis | Automnalis | 1 |
| Bataviae | Bataviae | 1 |
| Sejroe | Sejroe | 1 |
| Ballum | Castellonis | 1 |
| Shermani | Shermani | 1 |
| Pomona | Pomona | 1 |
| Semaranga | Patoc | 1 |

The infecting serovar: the only serovar reacted to MAT or servovar with highest titer

However, according to the type of habitat, positive MAT rate register in rats captured in urban area ($32^{+}$/63) seemed to be higher than the rate register in rats captured in rural area ($11^{+}$/37) (P=0.04) (Table 5).

## Culture results

From the 100 rats, 8 rats were positive by EMJH's culture for at least one organ (kidney or liver) including five *Rattus norvegicus* and three *Rattus rattus*. The prevalence was thus 8% (95% CI: 2.68%-13.32%), among which, 4 (4%) rats had positive kidney culture (all were *R. norvegicus*), 2 (2%) had positive liver culture (both were *R. rattus*) and finally, 2 (2%) rats had both organs positive culture including one *R. rattus* and one *R. norvegicus*.

**Table 4:** Number of reactions to multiple serovars (number of sera 23)

| Serogroups | Serovars | Number of reactions |
|---|---|---|
| Australis | Australis | 00 |
| Autumnalis | Autumnalis | 01 |
| Bataviae | Bataviae | 05 |
| Canicola | Canicola | 09 |
| Ballum | Castellonis | 00 |
| Cynopteri | Cynopteri | 02 |
| Grippotyphosa | Grippotyphosa | 02 |
| Hebdomadis | Hebdomadis | 00 |
| Sejroe | Sejroe | 04 |
| | Hardjo | 00 |
| Icterohaemorrhagiae | Icterohaemorrhagiae | 05 |
| | Icterohaemorrhagiae verdun | 11 |
| Panama | Panama | 06 |
| Louisiana | Louisiana | 02 |
| Pomona | Pomona | 04 |
| Pyrogenes | Pyrogenes | 04 |
| Tarassovi | Tarassovi | 04 |
| Celledoni | Celledoni | 00 |
| Djasiman | Djasiman | 00 |
| Mini | Mini | 05 |
| Sarmin | Sarmin | 06 |
| Shermani | Shermani | 01 |
| Javanica | Javanica | 00 |
| Semaranga | Patoc | 03 |
| Total | | 74 |

Among the eight positive rat tissue cultures, three (3/8) were negative by MAT and five (5/8) were positive, among their sera, 3 reacted to Icterohaemorrhagiae serougroup, 1 to seroupgoup Mini and 1 to both serogroups Canicola and Bataviae.

**Table 3:** Antibody maximum and minimum titers to various *Leptospira* serovars in the serum of rats, as assessed by MAT

| Serogroup | Serovar | Number of positive reactions | Titres Min-Max |
|---|---|---|---|
| Australis | Australis | 3 | 40-320 |
| Autumnalis | Autumnalis | 2 | 20-40 |
| Bataviae | Bataviae | 6 | 20-40 |
| Canicola | Canicola | 9 | 160-2560 |
| Ballum | Castellonis | 1 | 20 |
| Cynopteri | Cynopteri | 2 | 20 |
| Grippotyphosa | Grippotyphosa | 4 | 20-80 |
| Hebdomadis | Hebdomadis | 0 | 0 |
| Sejroe | Sejroe | 4 | 20-320 |
| | Hardjo | 0 | 0 |
| Icterohaemorrhagiae | Icterohaemorrhagiae | 7 | 20-80 |
| | Icterohaemorrhagiae verdun | 14 | 20-640 |
| Panama | Panama | 8 | 20-40 |
| Louisiana | Louisiana | 3 | 20-640 |
| Pomona | Pomona | 4 | 20-40 |
| Pyrogenes | Pyrogenes | 4 | 20-80 |
| Tarassovi | Tarassovi | 4 | 20-80 |
| Celledoni | Celledoni | 0 | 0 |
| Djasiman | Djasiman | 0 | 0 |
| Mini | Mini | 6 | 20-80 |
| Sarmin | Sarmin | 8 | 20-40 |
| Shermani | Shermani | 2 | 20-40 |
| Javanica | Javanica | 0 | 0 |
| Semaranga | Patoc | 3 | 80-160 |
| Total | / | 94 | / |

**Table 5:** Chi-square analysis of risk factors associated with *Leptospira* seroprevalence

| Independent variable | Categories | Total number of captured rats | Total number of positive rats | Percentage of positive rats (%) | Confidence interval (95% CI) | P-value |
|---|---|---|---|---|---|---|
| Sex | Female | 53 | 22 | 41.51 | 28.21-54.81 | 0.75 |
| | Male | 47 | 21 | 44.68 | 30.48-58.89 | |
| Age | Adult | 73 | 32 | 43.83 | 32.43-55.23 | 0.78 |
| | Sub-adult | 27 | 11 | 40.74 | 21.97-59.24 | |
| Rat's species | *Rattus Norvegicus* | 88 | 37 | 42.06 | 31.76-52.36 | 0.60 |
| | *Rattus Rattus* | 12 | 6 | 50 | 21.7-78.3 | |
| Habitat | Urban | 63 | 32 | 50.79 | 38.45-63.13 | 0.04 |
| | Rural | 37 | 11 | 29.73 | 15-44.46 | |
| Total | | 100 | 43 | / | / | / |

# Discussion

## Microscopic agglutination test

Molecular tools present a high sensitivity and specificity (Hamond *et al*., 2014) and have the advantage to detect *Letospira* DNA without requiring a viable organism and even before the serological reaction has been set (Waggoner and Pinsky, 2016). However, MAT can give a general impression of the serogroups/serovars circulating among populations; therefore, it is considered as the suitable test to use in a sero-epidiomiological surveys (Levett, 2001).

Among 100 captured rats, 43 were positive by MAT for at least one serovar, the seroprevalence was thus 43% (95% CI: 33.3%-52.7%). Comparing our seroprevalence to those reported in rats in some neighborhood region. Our seroprevalence is much higher than the one conducted in Tunisia (7.3%) (Lazuga and Bonnefous, 1962) and in Mahala city, Egypt 14% (Felt *et al*., 2011). However, it is lower than those reported in Nigeria on *Rattus Norvegicus* (76.9%) (Udechukwu *et al*., 2021) and in another study conducted in Egypt on rats (75.9%) (Samir *et al*., 2015). The comparison of prevalence is however complicated because of the different laboratory methods admitted in each study, the sample size used, the climatic conditions and the different cut off used for MAT.

The serogroup Icterohaemorrhagiae has been found to be the most prevalent infecting serogroup 11% (11/100). This finding confirms the role of rats as the main reservoir for this serogroup (Bharti *et al*., 2003; Vinetz *et al*., 2005), moreover, Icterohaemorrhagiae is a serougroup found to be responsible of the majority of human leptospirosis cases (Picardeau, 2013) and was also the dominant serogroup detected in human cases in Algeria (Afiri, 2013; Afiri *et al*., 2013). In addition to serology, Icterohaemorrhagiae has also been identified in *Rattus* species by molecular tools, in France, using multi-spacer sequence typing (MST) (Ayral *et al*., 2015; Garcia-Lopez *et al*., 2024) and in Serbia (Gajdov *et al*., 2024) using multi-locus sequence typing (MLST).

The second predominant serogroup was Canicola. According to the literature, dogs are likely its common reservoir (Bharti *et al*., 2003; Perez-Garcia *et al*., 2022), this observation makes us believe that there is a close cohabitation between dogs and rats in the study area.

Most of the serums reacted to more than one serovar, with some reacted to Patoc serovar. In fact, the serovars used for MAT in our study are not locally isolated; knowing that, the locally isolated strain increase for best the sensitivity of the test (Levett, 2001).

No statistical difference was recorded in antibodies' prevalence between female and male rats (P=0.75), our results are in agreement with the studies conducted on rats in Vietnam and in Iran (Bahman *et al*., 2013; Loan *et al*., 2015). No difference in the seo-prevalence was register too between adult and sub-adult rats (P=0.78). In fact, rats live hierarchically in colonies (Schweinfurth, 2020). As a result, both sexes are similarly exposed to *Leptospira* infection (Sharma *et al*., 2019), as well as all rats of different age.

*Rattus norvegicus* were as infected as *R. nattus* (P=0.41). However, according to Boey *et al*. (2019), seroprevalece of *Leptospira* spp. in *R. norvegicus* is generally higher than in *R. nattus* worldwide. In our study, only twelve *R. nattus* were captured, the small simple size of captured *R. nattus* is therefore insufficient to confirm our finding.

According to Chi-square analysis, rats captured in urban areas were more infected than those captured in rural area (P=0.04), effectively. The high density of rats in urban areas associated with stagnant water could facilitate the spread of leptospires among rats (Boey *et al*., 2019).

## *Leptospira* culture

Culture can allow a definitive identification of the infecting serovar (Herbreteau *et al*., 2011); however, the preparation of the medium is technically difficult and expensive (Bharti *et al*., 2003) with low sensitivity rate (Limmathurotsakul *et al*., 2012). In addition, getting positive cultures in natural infected specimens is difficult because of the presence of microorganisms contaminating the samples and the need of time between sampling and laboratory processing (Thiermann, 1984). Moreover, to increase the rate of positivity, the ideal was to use two types of culture for each sample, one with antibiotic and the other without antibiotic (Kososssey-Vrain, 2004). Furthermore, three decimal dilutions of the initial culture should be incubated (1/10, 1/100 and 1/1000) (WHO, 2003); however, as mentioned before, such protocol is technically expensive to realize. In our study, the choice was to not use antibiotic since antibiotic can in parallel reduce the growth of *Leptospira*

(WHO, 2003); the contamination was therefore controlled by passing the culture medium throw filter of 0.45 µm and 0.22 µm (Postic *et al*., 2000). Besides, for each tissue's culture, only one decimal dilution (1/10) was unfortunately used.

In our study, *Leptospira* was isolated from kidneys and livers of 8 rats. However, to confirm the shedding status, culture should be done on urine samples since when, leptospires are excreted in urine intermittently (Athapattu *et al*., 2019), luckily, a later study was conducted by real-time PCR in the same area, the study has confirmed the carrying and the shedding status of rats with a prevalence of 40.6% (Lekhal *et al*., 2022).

Among the 8 positive cultures, 3 were negative by MAT. The seronegativity associated to positive culture specimens suggests that rats chronically infected with *L. interrogans* may become negative by serology (Di Azevedo and Lilenbaum, 2021), or, simply, the infecting serovar was not included in the panel of the MAT test (Scialfa *et al*., 2013). However, for the rest of positive cultures (5/8), the MAT titers were proportionally low (from 20 to 320), effectively, the circulating antibodies do not always correspond to the strain of *Leptospira* present in kidneys (Villanueva *et al*., 2010).

For the best of our knowledge, this is the first study highlighting the importance of rats as reservoir host of *Leptospira* pathogen by serology and by culture in the city of Blida; Algeria. The serogroup Icterohaemorrhagiae was the predominant infecting serovar.

Leptospires were isolated from kidney and or liver confirming; therefore, the carrying status of rats in the study area.

Further surveys should be done targeting other animal species and other areas of the country by using molecular tools such real-time PCR, sequencing and genotyping to understand for better the epidemiology of the transmission of the bacteria and to evaluate the risk of transmission to human for the adaptation of an effective prophylactic measures.

## Acknowledgements

The authors would like to thank the Service of Hygiene of Blida and particularly Dr. A. Ouchfoun, the Chief Service for the help provided during the rodents trapping.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## References

**Adler, B and de laPeña Moctezuma, A** (2010). *Leptospira* and leptospirosis. Vet. Microbiol., 140: 287-296.

**Afiri, M** (2013). Renal manifestations of leptospirosis: 88 cases. Med. Sante Trop., 23: 234-235.

**Afiri, M; Toudeft, F and AitKaid, D** (2013). Leptospirosis epidemic: 48 cases. Med. Sante Trop., 23: 234. https://doi.org/10.1684/mst.2013.0203.

**Ahmad, SN; Shah, S and Ahmad, FMH** (2005). Laboratory diagnosis of leptospirosis. J. Posgrad. Med., 51: 195-200.

**Ahmim, M** (2004). Mammals of Algeria. From the Origins to the Present Day. Ministry of Territorial Planning and Environment, Algiers, Algeria. PP: 196-200.

**Athapattu, TPJ; Fernando, BR; Koizumi, N and Gamage, CD** (2019). Detection of pathogenic leptospires in the urine of domesticated elephants in Sri Lanka. Acta Trop., 195: 78-82.

**Ayral, F; Zilber, AL; Bicout, DJ; Kodjo, A; Artois, M and Djelouadji, Z** (2015). Distribution of *Leptospira interrogans* by multispacer sequence typing in urban Norway rats (*Rattus norvegicus*): A survey in France in 2011-2013. PLoS One. 10: e0139604. https://doi.org/10.1371/journal.pone.0139604.

**Bahman, M; Masoud, GN; Reza, A and Gholamreza, A** (2013). A serological survey on leptospiral infection among wild rats (*Rattus rattus*) of Ahvaz district, southwest of Iran: A preliminary study. Jundishapur J. Microbiol., 6: 66-71.

**Benseghir, H; Amara-Korba, A; Azzag, N; Hezil, D and Ghalmi, F** (2020). Seroprevalence of and associated risk factors for *Leptospira interrogans* serovar *hardjo* infection of cattle in Setif, Algeria. Afr. J. Clin. Exp. Microbiol., 21: 185-191.

**Bharti, AR; Nally, JE; Ricaldi, JN; Matthias, MA; Diaz, MM; Lovett, MA; Levett, PN; Gilman, RH; Willig, MR and Gotuzzo, E** (2003). Leptospirosis: A zoonotic disease of global importance. Lancet Infect. Dis., 3: 757-771.

**Boey, K; Shiokawa, K and Rajeev, S** (2019). *Leptospira* infection in rats: A literature review of global prevalence and distribution. PLoS Negl. Trop. Dis., 13: e0007499. https://doi.org/10.1371/journal.pntd.0007499.

**Caimi, K and Ruybal, P** (2020). *Leptospira* spp., a genus in the stage of diversity and genomic data expansion. Infect. Genet. Evol., 81: 104241. https://doi.org/10.1016/j.meegid.2020.104241.

**Costa, F; Hagan, JE; Calcagno, J; Kane, M; Torgerson, P; Martinez-Silveira, MS; Stein, C; Abela-Ridder, B and Ko, AI** (2015). Global morbidity and mortality of leptospirosis: A systematic review. PLoS Negl. Trop. Dis., 9: e0003898. https://doi.org/10.1371/journal.pntd.0003898.

**Derdour, SY; Hafsi, F; Azzag, N; Tennah, S; Laamari, A; China, B and Ghalmi, F** (2017). Prevalence of the main infectious causes of abortion in dairy cattle in Algeria. J. Vet. Res., 61: 337-343.

**Di Azevedo, MIN and Lilenbaum, W** (2021). An overview on the molecular diagnosis of animal leptospirosis. Lett. Appl. Microbiol., 72:496-508.

**Felt, SA; Wasfy, MO; El-Tras, WF; Samir, A; Abdel Rahaman, B; Boshra, M; Parker, TM; Hatem, ME; El-Bassiouny, AA; Murray, CK and Pimentel, G** (2011). Cross-species surveillance of *Leptospira* in domestic and peri-domestic animals in Mahalla city, Gharbeya Governorate, Egypt. Am. J. Trop. Med. Hyg., 83: 420-425.

**Gajdov, V; Jokic, G; Savic, S; Zekic, M; Blazic, T; Rajkovic, M and Petrovic, T** (2024). Genotyping of *Leptospira* spp. in wild rats leads to first time detection of *L. kirshneri* serovar Mozdok in Serbia. Front. Microbiol., 28: 1379021. https://doi.org/10.3389/fmicb.2024.1379021.

**Garcia-Lopez, M; Lurier, T; Bouilloud, M; Pradel, J; Tatard, C; Sepulveda, D; Anfray, G; Dussert, J; Bourhy, P; Charbonnel, N and Djelouadji, Z** (2024). Prevalence, genetic diversity and eco-epidemiology of

pathogenic *Leptospira* species in small mammal communities in urban parks Lyon city, France. PLoS One. 19: e0300523. https://doi.org/10.1371/journal.pone. 0300523.

**Geisen, V; Stengel, C; Brem, S; Muller, W; Greene, C and Hartmann, K** (2007). Canine leptospirosis infections: clinical signs and outcome with different suspected *Leptospira* serogroups (42 cases). J. Small Anim. Pract., 48*:* 324-328.

**Hamond, C; Martins, G; Loureiro, AP; Pestana, C; Lawson-Ferreira, R; Medeiros, MA and Lilenbaum, W** (2014). Urinary PCR as an increasingly useful tool for an accurate diagnosis of leptospirosis in livestock. Vet. Res. Commun., 38: 81-85.

**Herbreteau, V; Jittapalapong, S; Rerkamnuaychoke, W; Chaval, Y; Cosson, JF and Morand, S** (2011). Protocols for field and laboratory rodent studies. Kasetsart University, Bangkok, Thailand. PP: 20-23.

**Kososséy-Vrain, C** (2004). Canine leptospirosis: bibliographical review. Med. Vet. ENVA. 135: 77-94.

**Lazuga, K and Bonnefous, S** (1962). Contribution to the study of leptospirosis in rats in the city of Tunis. Arch. Past. Instit. Tunis. 39: 49-65.

**Lekhal, L; Harran, E; Aragon, A; Groud, K; Le Guyader, M; Kaidi, R; Khelef, D and Djelouadji, Z** (2022). First molecular detection of pathogens *Leptospira* in common rodent captured in north Algeria urban areas. Trop. Med. Infect. Dis., 7: 335. https://doi.org/10.3390/tropicalmed 7110335.

**Levett, PN** (2001). Leptospirosis. Clin. Microbiol. Rev., 14: 296-326.

**Limmathurotsakul, D; Turner, EL; Wuthiekanun, V; Thaipadungpanit, J; Suputtamongkol, Y; Chierakul, W; Smythe, LD; Day, NP; Cooper, B and Peacock, SJ** (2012). Fool's gold: Why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. Clin. Infect., 55: 322-331.

**Loan, HK; Cuong, NV; Takhampunya, R; Kiet, BT; Campbell, J; Them, LN; Bryant, JE; Tippayachai, B; Hoang, NV; Morand, S; Hien, VB and Carrique-Mas, JJ** (2015). How important are rats as vectors of leptospirosis in the Mekong Delta of Vietnam? Vector-Borne Zoonotic Dis., 15: 56-64.

**Musso, D and La Scola, B** (2013). Laboratory diagnosis of leptospirosis: A challenge. J. Microbiol. Immunol. Infect., 46: 245-252.

**Noh, MA; Masri, SN; Zulkapli, A; Mohd Ali, MR and Amran, F** (2024). *Leptospira* infection and carrier survey on rats from wet market areas in Kuala Lumpur, Malaysia. J. Vector Borne Dis., 61: 43-50.

**O'Keefe, JS; Jenner, JA; Sandifer, NC; Antony, A and Williamson, NB** (2002). A serosurvey for antibodies to *Leptospira* in dogs in the lower North Island of New Zealand. N. Z. Vet. J., 50: 23-25.

**Perez-Garcia, J; Monroy, FP and Agudelo-Florez, P** (2022). Canine leptospirosis in a northwestern region of Colombia: serological, molecular and epidemiological factors. Pathogens. 11: 1040. https://doi.org/10.3390/pathogens 11091040.

**Picardeau, M** (2013). Diagnosis and epidemiology of leptospirosis. Médecine et Maladies Infectieuses. 43: 1-9.

**Postic, D; Merien, F; Perolat, P and Baranton, G** (2000). *Biological diagnosis leptospirosis - Lyme borreliosis*. 2nd Edn., Paris, Collection of Reference and Expertise Laboratories. Pasteur Institute in Paris. PP: 177-186.

**Samir, A; Soliman, RK; El-Hariri, M; Abdel-Moein, K and Essam Hatem, M** (2015). Leptospirosis in animals and human contacts in Egypt: broad range surveillance. Rev. Soc. Bras. Med. Trop., 48: 272-277.

**Samrot, AV; Sean, TC; Bhavya, KS; Sahithya, CS; Chan-Drasekaran, S; Palanisamy, R; Robinson, ER; Subbiah, SK and Mok, PL** (2021). Leptospiral infection, pathogenesis and its diagnosis-a review. Pathogens. 10: 145. https://doi.org/ 10.3390/pathogens10020145.

**Schweinfurth, MK** (2020). The social life of Norway rats (*Rattus norvegicus*). elife. 9: e54020. https://doi.org/10. 7554/eLife.54020.

**Scialfa, E; Giamperetti, S; Brihuega, B; Grune, S; Aguirre, P and Gallicchio, O** (2013). Leptospirosis in synanthropic animals captured in peri-urban areas of the city of Azul, Province of Buenos Aires. 4th Congress on Endoepidemic Diseases, Muñiz Hospital, Buenos Aires. Rev. Infectol. Dr. Francisco J. Muñiz, 16: 32.

**Sharma, RN; Thille, K; Piechowski, B and Tiwari, K** (2019). Detection of serum antibodies against *Leptospira* spp. in brown rats (*Rattus norvegicus*) from Grenada, West Indies. Vet. World. 12: 696-699.

**Thiermann, AB** (1984). Isolation of leptospires in diagnosis of leptospirosis. Mod. Vet. Pract., 5: 758-759.

**Thierry, A** (2012). *Statistical epidemiology*. 3rd Edn., Second Printing, Series Edited by D'Ivernois, JF, Maloine. PP: 77-121.

**Tilley, LP; Smith, Jr and Francis, WK** (2004). *The 5-minute veterinary consult*. 3rd Edn., Philadelphia, Lippincott Williams & Wilkins. PP: 768-769.

**Udechukwu, CC; Kudi, CA; Abdu, PA; Abiayi, EA and Orakpoghenor, O** (2021). Prevalence of *Leptospira interrogans* in wild rats (*Rattus norvegicus* and *Cricetomys gambianus*) in Zaria, Nigeria. Heliyon. 7: e05950. https://doi.org/10.1016/j.heliyon.2021.e05950.

**Vanasco, NB; Lottersberger, J; Sequeira, MD and Tarabla, H** (2001). Development and validation of an ELISA for the detection of leptospire-specific antibodies in rodents. Vet. Microbiol., 82: 321-330.

**Villanueva, S; Ezoe, H; Baterna, RA; Yanagihara, Y; Muto, M; Koizumi, N; Fukui, T; Okamoto, Y; Masuzawa, T; Cavinta, LL; Gloriani, NG and Yoshida, S** (2010). Serologic and molecular studies of *Leptospira* and leptospirosis among rats in the Philippines. Am. J. Trop. Med. Hyg., 82: 889-898.

**Vinetz, JM; Wilcock, BA; Aguirre, A; Gollin, LX; Katz, AR; Fujioka, RS; Maly, K; Horwitz, P and Chang, H** (2005). Beyond disciplinary boundaries: leptospirosis as a model of incorporating transdisciplinary approaches to understand infectious disease emergence. EcoHealth. 2: 291-306.

**Waggoner, JJ and Pinsky, BA** (2016). Molecular diagnostics for human leptospirosis. Curr. Opin. Infect. Dis., 29: 440-445.

**World Health Organization (WHO)** (2003). Human leptospirosis: guidance for diagnosis, surveillance and control, Geneva. Available at: http://www.who.int/csr/don/ en/WHO_CDS_CSR_EPH_2002.23.pdf.

**Zaidi, S; Bouam, A; Bessas, A; Hezil, D; Ghaoui, H; Ait-Oudhia, K; Drancourt, M and Bitam, I** (2018). Urinary shedding of pathogenic *Leptospira* in stray dogs and cats, Algiers: A prospective study. PLoS One. 13: e0197068. https://doi.org/10.1371/journal.pone.0197068.