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Serological study of leptospirosis in cats from Algeria

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Abstract:

Background: By the nature of their environment and behavior, stray cats are at risk of exposure to leptospirosis. Leptospirosis is an emerging zoonotic disease with worldwide distribution. The prevalence of leptospirosis in the feline species in Algeria is unknown. The main objectives of this study are to determine the seroprevalence and identify the most common *Leptospira* serovars in stray cats in the Algiers region.
Methodology: Serum samples from 144 randomly selected healthy stray cats from 57 municipalities of the Algiers region were analyzed by the microscopic agglutination test (MAT). The MAT was performed to determine the antibody titers against nine *Leptospira* serovars (Canicola, Copenhageni, Icterohaemorrhagiae, Autumnalis, Grippotyphosa, Bratislava, Pomona, Pyrogenes, Patoc). The age of each cat was estimated based on dentition and physical appearance, and information on cat sex, breed and clinical status were collected. Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 17.0
Results: *Leptospira* antibodies were detected in 8 of 144 healthy stray cats, giving a seroprevalence rate of 5.6% [95% confidence interval (CI)=1.814-9.297]. The antibody titers ranged from 1:100 to 1:3200. Serovars Pyrogenes (1:100) and Patoc (1:100) were the most prevalent serovars detected in 2.8% (4/144) of the cats, followed by serovars Icterohaemorrhagiae (1:100) and Bratislava (1:100) detected in 2.1% (3/144) of the cats. The seroprevalence of 7.8% (7/90) in the male cats was higher than 1.9% (1/54) in the female cats but this did not reach a significant difference (OR=4.47, 95% CI=0.5344-37.387, p=0.2586). All the positive cats were over one year of age.
Conclusion: This study showed that stray cats in Algiers are exposed to leptospirosis. In addition, the serovars detected are very common serovars in dogs and humans. The control of leptospirosis is largely dependent on general hygiene measures and the control of animal reservoirs. Additional investigations are necessary to clarify the epidemiology of the disease in the different regions of Algeria.

Keywords: *Leptospira*, cats, serology, MAT, Algiers

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Etude sérologique de la leptospirose chez les chats d'Algérie

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Résumé:

Contexte: De par la nature de leur environnement et de leur comportement, les chats errants sont à un risque d'exposition à la leptospirose. La leptospirose est une maladie zoonotique émergente de distribution mondiale. La prévalence de la leptospirose chez l'espèce féline en Algérie est inconnue. Les principaux objectifs de cette étude sont de déterminer la séroprévalence et d'identifier les sérovars de *Leptospira* les plus fréquents chez les chats errants de la région d'Alger.
Méthodologie: Des échantillons de sérum de 144 chats errants sanitaires sélectionnés au hasard dans 57 communes de la région d'Alger ont été analysés par le test d'agglutination microscopique (MAT). Le MAT a été réalisé pour déterminer les titres d'anticorps contre neuf sérotypes de *Leptospira* (Canicola, Copenhageni, Icterohaemorrhagiae, Autumnalis, Grippotyphosa, Bratislava, Pomona, Pyrogenes, Patoc). L'âge de chaque chat a été estimé sur la base de la dentition et de l'apparence physique, et des informations sur le sexe, la race et l'état clinique du chat ont été collectées. Les données ont été analysées à l'aide du package statistique pour les sciences sociales (SPSS) version 17.0
Résultats: Des anticorps contre *Leptospira* ont été détectés chez 8 des 144 chats errants sanitaires, donnant un taux de séroprévalence de 5,6% [intervalle de confiance (IC) à 95%=1,814-9,297]. Les titres d'anticorps variaient de 1:100 à 1:3200. Les sérovars Pyrogenes (1:100) et Patoc (1:100) étaient les sérovars les plus prévalents détectés chez 2,8 % (4/144) des chats, suivis des sérovars Icterohaemorrhagiae (1:100) et Bratislava (1:100) détectés chez 2,1% (3/144) des chats. La séroprévalence de 7,8 % (7/90) chez les chats mâles était supérieure à 1,9 % (1/54) chez les chattes, mais cela n'a pas atteint une différence significative (OR=4,47, IC 95%=0,5344-37,387, p=0,2586). Tous les chats positifs avaient plus d'un an.
Conclusion: Cette étude a montré que les chats errants d'Alger sont exposés à la leptospirose. De plus, les sérovars détectés sont des sérovars très répandus chez le chien ou chez l'homme. Le contrôle de la leptospirose est largement tributaire des mesures d'hygiène générales et de la lutte contre les réservoirs animaux. Des investigations complémentaires sont nécessaires pour préciser l'épidémiologie de la maladie dans les différentes régions de l'Algérie.

Mots clés: *Leptospira*, chats, sérologie, MAT, Alger

Introduction:

Leptospirosis is a global disease affecting many domestic and wild animal species, and is considered a zoonotic disease. It causes serious problems in tropical and temperate climates whether in urban or rural environments (1). This zoonosis is caused by pathogenic spirochetes of the genus *Leptospira* which colonize the renal tubules where they reproduce before being excreted in the urine (2). Infected urine or contaminated water are sources of leptospirosis and *Leptospira* can enter the body of mammalian hosts through lacerations of skin, mucous membranes, conjunctiva and inhalation of aerosols (3,4).

Previously, domestic cats were thought to be resistant to leptospirosis and many practitioners do not consider feline leptospirosis in the differential diagnosis with other diseases (5). However, recently published reports on feline leptospirosis concluded that cats are exposed to *Leptospira* and may play a role in the epidemiology of this disease (6,7). The presence of viable pathogenic Leptospires in the urine of cats has been proven (8,9). Therefore, the species can play a role in the transmission of the zoonosis.

Our previous study in Algiers was molecular and did not demonstrate carriage and urinary excretion of pathogenic Leptospires in cats (10). According to Hartmann et al., (11), renal carriage and leptospiruria in naturally infected cats may have been underestimated. Rodents are known to serve as the main reservoir of pathogenic *Leptospira*. Thus, the number of infected cats could also be high, as rodent hunting is believed to be the main source of infection in cats (11). Infection from water or urine of cohabiting dogs seems to play a minor role in cats (11). In Algeria, leptospirosis poses a real public health problem and every year there are human cases that occur however, animal studies are limited in the region (10,12-15). Cats are becoming increasingly popular as pets in Algiers and therefore it is important to have data to assess at what rate cats pose a risk for human leptospiral infection.

The aims of the present study were to determine the seroprevalence of *Leptospira* and the most prevalent serovars in stray cats in the region of Algiers, using the most common serological test for the diagnosis of leptospirosis, micro-agglutination test (MAT).

Materials and method:

Study design and sampling

In order to carry out a serological survey and detect the incriminated serovars, 144 stray cats were randomly selected from the 57 municipalities of Algier region. About 5 to 10 ml of venous blood were collected in dry tubes. The sera were obtained by centrifugation for 5 to 10 minutes at 3000 rpm and then stored at −20°C until serological tests were performed. The age of each cat was

estimated based on dentition and physical appearance. Information regarding the sex, breed and clinical status was recorded.

Ethical statement

The study was approved by the ethics committee and decision board (number 416/2017) of Entreprise Publique à Caractère Industriel et Commercial - Hygiene Urbaine et Protection de l'Environnement (EPIC-H.U.P.E) of Wilaya of Algiers (Ex: HURBAL).

Microscopic agglutination test

The microscopic agglutination test (MAT) with 92% sensitivity and 60-100% specificity is regarded as the 'gold standard' method for the diagnosis of leptospirosis, and detects different serovars (16). The MAT was performed according to the Office International des Epizooties (OIE) standards 2008. The MAT was carried out at *Leptospira* unit, Pasteur Institute of Algiers, Algeria, and antibody titres were determined against nine *Leptospira* serovars (Canicola, Copenhageni, Icterohaemorrhagiae, Autumnalis, Grippotyphosa, Bratislava, Pomona, Pyrogenes, and Patoc) (Table 1). The MAT titre equal to or higher than 1:100 was considered positive.

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

Serogroups	Serovars	Strains
Canicola	Canicola	Hond Utrecht
Icterohaemorrhagiae	Copenhageni	Willinberg
Icterohaemorrhagiae	Icterohaemorrhagiae	Verdun
Autumnalis	Autumnalis	Akiyami A
Grippotyphosa	Grippotyphosa	Moskva V
Australis	Bratislava	Jez Bratislava
Pomona	Pomona	Pomona
Pyrogenes	Pyrogenes	Salinem
Semaranga	Patoc	Patoc1

Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL). Association of variables (sex, age, breed, clinical signs) with the seroprevalence of leptospirosis was done using Chi-square ($\chi 2$) test (Yates corrected). P values lower than 0.05 were considered as indicative of significance.

Results:

Microscopic agglutination test result

Antibodies were detected samples of 8 of the 144 cats, representing a sero-prevalence of 5.6% [95% confidence interval (CI) =1.814-9.297]. All the 8 cats tested positive to at least one *Leptospira interrogans* serovar at a dilution of ≥ 1:100. Serovars Pyrogenes (1:100) and Patoc (1:100) were the most prevalent, detected in 2.8% (4/144) of the cats, followed by serovars Icterohaemorrhagiae (1:100) and Bratislava (1:100), detected in 2.1% (3/144) of the cats. One serovar each was detected in 3 cats, two serovars each in 4 cats and three serovars in 1 cat (Table 2).

Characteristics and risk factors associated with seropositivity to *Leptospira*

All the stray cats sampled were cats of common breed, hence all the 8 cats seropositive for *Leptospira* were common breed. There were 90 (62.5%) male and 54 (37.5%) female cats; 112 (77.8%) cats were over one year while 32 (22.2%) cats were under one year old. The seroprevalence of 7.8% (7/90) in the male cats was higher than 1.9% (1/54) in the female cats but this did not reach a significant difference (OR = 4.47, 95% CI = 0.5344-37.387, p=0.2586). All the stray cats seropositive for *Leptospira* were over 1 year old. Also, all the stray cats sampled were healthy and did not show any clinical signs of disease (Table 3).

Table 4 shows published articles on leptospirosis from other countries over the last five years (17-24), with seroprevalence rates reported in cats varying between 0% and 42%. The table provides infirmation on study area, period of study, way of life, clinical status, number of serum samples collected, positive results, predominant serovars or serogroups, and cut-off values used in each study.

Discussion:

In the present study, we evaluated the prevalence of *Leptospira* infection among cats in Algiers regions of Algeria. Serological method by MAT was used in order to determine the infectious status of the 144 stray cats. Looking at studies carried out in other countries published over the last five years, the seroprevalence observed in cats varies between 0% and 42% (17-24). Our study records a low seroprevalence of 5.6% in stray cats (95%CI=1.814-9.297). As there is no systematic vaccination program of the feline population against leptospirosis in Algiers, all the more reason that these animals live outside, these positive sera can be considered as active or previous infections. The lack of vaccine against leptospirosis for cats is justified by the low morbidity of the disease. Therefore, seropositivity is a true indicator of exposure and cannot be confused with post-vaccination seropositivity (23).

Our prevalence rate is similar to that reported in several studies (18,20,25) except for some studies where the prevalence was higher due to the cut-off value of 1:50 used (22-24). In cats, antibody levels are commonly low and often lower than those in other animals (26-29). Environmental factors such as outdoor habits, presence of farm

animals that may shed Leptospires in the neighborhood, hunting habits, or even the season of the year, can explain the broad ranges of antibody prevalence reported in the literature (20). Reports have shown that leptospirosis prevalence can differ not only according to country, but to particular region also (30), even as different cut off values (≥1:100) and serovar panels used in laboratories may affect the prevalence. There is no consensus on the most appropriate cut-off value to choose in cats, and cats are thought to respond to infection with low antibody titers, ranging from 1:30 to 1:400, as has been demonstrated in experimental and natural infections (22). The results of our study suggest that stray cats in Algiers are in contact with pathogenic Leptospires, probably through other maintenance host species such as rodents.

Table 2: Antibody titers to various *Leptospira* serovars in the serum of stray cats from Algiers, as assessed by MAT

Serovars / **Cases**	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Case 7**	**Case 8**
Canicola	-	-	-	-	-	-	-	-
Copenhageni	-	-	-	-	-	-	-	-
Icterohaemorrhagiae	-	-	1600	-	-	100	1600	-
Autumnalis	-	-	-	-	-	-	-	-
Grippotyphosa	-	-	-	-	-	-	-	-
Bratislava	-	-	-	100	200	-	-	100
Pomona	-	-	-	-	-	-	-	-
Pyrogenes	100	200	3200	-	100	-	-	-
Patoc	100	100	-	100	200	-	-	-

MAT = Microscopic agglutination test

Table 3: Seroprevalence and univariable analysis of risk factors associated with *Leptospira interrogans* among sampled cats

Independent Variables	**Categories**	**Number of cats sampled**	**Total number of positive results (%)**	**OR (95% CI)**	***p* value**
Breed	Common breed	144	8 (5.6)	-	-
Sex	Female	54	1 (1.9)	4.47 (0.5344-37.387)	0.2586[+]
	Male	90	7 (7.8)		
Age (years)	< 1 year	32	0	0.1891 (0.01062-3.369)	0.1996[+]
	> 1year	112	8 (7.1)		
Clinical sign		144	8 (5.6%)	-	-
Total			144		

OR=Odds ratio; CI=Confidence interval; + = not statistically significant

In the present study, cross-reactions between two or more serogroups were present in all cases. Serovars Pyrogenes and Patoc were the most detected servars, followed by Icterhemorrhagiae and Bratislava. It is possible that cats were infected with serovars not included in the panel or unknown serovars. The simultaneous seropositivity exhibited by some cats, not only in our study but also in several other studies, could be explained either by true cross-reactivity in the cats, or by simultaneous exposure of animals to different serovars (20). The high titers observed for the serovar Icterohemorrhagiae (1:1600) in this study, would suggest that rats are the source of infection for some cats. Serovar Canicola was not detected in this study, knowing that it is the major serovar, as far as canine leptospirosis is concerned. It has been already reported that serovars Icterohemorrhagiae, Canicola, Grippotyphosa, Pomona and Bratislava are the most common *Leptospira* serovars isolated from cats (Table 4). However, the range of serovars should not be limited to local strains as the infection may be caused by a rare serovar or a strain not previously described.

We included the saprophytic strains (Patoc) in our diagnostic panel, which can cross-react with antibodies produced by certain pathogenic serovars (20). Four of the 8 cats with antibodies against pathogenic *Leptospira* did not have antibodies against saprophytic serovars. This can be explained by the fact that infections are old and saprophytic serovars, in particular serovar Patoc, have limited ability to detect cross-reactions with antibodies from past infections (31). According to the guidelines of the International Leptospirosis Society, the range of serovars should not be limited to local strains and thus, serovar Patoc should be included, because it cross-reacts with human or animal antibodies generated by a number of pathogenic serovars (18).

None of the seropositive cats presented with clinical signs compatible with leptospirosis such as fever, weight loss, jaundice, lethargy, ascites, renal failure or hepatitis (19). In addition to low serological response, cats are reported to rarely develop clinical leptospirosis (5,32,33). Nevertheless, clinical cases of leptospirosis have been reported in cats infected with higher titers (1:800) of serovar Pomona (19). Conclusively, cats do not seem clinically sensitive to *Leptospira* serotypes circulating in Algiers.

In our study, male cats were more seropositive than female cats, which is similar to what was reported in the study by Weis et al., (34), while the study by Bourassi et al., (22) noted that seropositive cats were more females than males. In many studies however, gender was not significantly associated with *Leptospira* seropositivity in cats (6,7,18,28,35-37). The seropositive cats in our study were all over one year old. Bourassi et al., (22) reported the same result that positive cats were more adults than juveniles. On the other hand, in the study by Milan et al., (27), seropositivity was more frequent in juvenile than in adult cats. Older age was already reported in other studies as a risk factor for *Leptospira* infection in cats (35-37).

Conclusion:

To the best of our knowledge, this is the first report of seroprevalence of pathogenic Leptospires in cats from Algiers by the MAT serological technique. In addition, the serovars detected are very frequent serovars in dogs and humans. Control of leptospirosis largely depends on general hygiene measures and control of animal reservoirs. Additional investigations are necessary to clarify the epidemiology of the disease in other animal species and in other regions of Algeria.

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Authors contributions:

ZS conceptualize the study, and was involved in methodology, visualization, and writing of the manuscript; AKA was involved in conceptualization, methodology, resources provision, supervision and visualization; BeA was involved in the methodology and manuscript writing; BoA was involved in methodology and visualization; HNK was involved in the methodology; HD.j was involved in the methodology and manuscript writing; and BI was involved in resources provision, supervision and visualization

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Conflict of interest:

Authors declare no conflict of interest

References:

1. Adler, B., and Moctezuma, A. P. Leptospira and leptospirosis. Vet Microbiol. 2010; 140: 287-296.
2. Levett, P. N. Leptospirosis. Clin Microbiol Rev. 2001; 14 (2): 296-326.

3. Baron, S. Medical Microbiology. University of Texas Medical Branch at Galveston. 1996: 1273
4. Musso, D., and La Scola, B. Laboratory diagnosis of leptospirosis: A challenge. J Microbiol Immunol Infect. 2013; 46 (4): 245-252.
5. Arbour, J., Blais, M. C., Carioto, L., et al. Clinical leptospirosis in three cats (2001–2009). J Am Anim Hosp Assoc. 2012; 48(4): 256-260.
6. Azócar-Aedo, L., Monti, G., and Jara, R. *Leptospira* spp. in Domestic Cats from Different Environments: Prevalence of Antibodies and Risk Factors Associated with the Seropositivity. Animals (Basel). 2014; 4(4): 612-626.
7. Rodriguez, J., Blais, M. C., Lapointe, C., et al. Serologic and urinary PCR survey of leptospirosis in healthy cats and in cats with kidney disease. J Vet Intern Med. 2014; 28(2): 284-293.
8. Dorsch, R., Salgado, M., Monti, G., et al. Urine shedding of pathogenic *Leptospira* spp. in cats in southern Chile in science for people. In: Proceedings of the 10th International Leptospirosis Society Conference, Palmerston North, New Zealand. 2017: 227.
9. Ojeda, J., Salgado, M., Encina, C., et al. Evidence of interspecies transmission of pathogenic Leptospira between livestock and a domestic cat dwelling in a dairy cattle farm. J Vet Med Sci. 2018; 80: 1305-1308.
10. Zaidi, S., Bouam, A., Bessas, A., et al. Urinary shedding of pathogenic *Leptospira* in stray dogs and cats, Algiers: A prospective study. PLoS One. 2018; 13 (5): e0197068.
11. Hartmann, K., Egberink, H., Pennisi, M. G., et al. *Leptospira* species infection in cats: ABCD guidelines on prevention and management. J Feline Med Surg. 2013; 15 (7): 576-581.
12. Derdour, S. Y., Hafsi, F., Azzag, N., et al. Prevalence of the main infectious causes of abortion in dairy cattle in Algeria. J Vet Res. 2017; 61: 337-343.
13. Yahiaoui, W. I., Amara-Korba, A., Aggad, H., et al. Seroprevalence of leptospirosis in some farms of Algiers (Algeria). Lucrari Stiintifice - Universitatea de Stiinte Agricole a Banatului Timisoara. Medicina Veterinar. 2018; 51 (3): 111-118.
14. Benseghir, H., Amara-Korba, A., Azzag, N., et al. Seroprevalence and risk factors for leptospirosis in cattle. Afr J Clin Exper Microbiol. 2020; 21 (3): 185-191.https://doi.org/10.4314/ajcem.v21i3.3
15. Benseghir, H., Hezil, Dj., Boucheml, F., et al. Sero-epidemiological study of *Leptospira interrogans* infection of cattle in north Algeria. Agricultura. 2021; 2: 117-118.
16. Sykes, J. E., Hartmann K., Lunn K. F., et al. 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. J Vet Intern Med. 2011; 25 (1) : 1-13.
17. Palerme, J. S., Lamperelli, E., Gagne, J., et al. Seroprevalence of *Leptospira* spp., *Toxoplasma gondii*, and *Dirofilaria immitis* in Free-Roaming Cats in Iowa. Vector Borne Zoonotic Dis. 2019; 19(3): 193-198.
18. Sprißler, F., Jongwattanapisan, P., Luengyosluechakul, S., et al. Leptospira infection and shedding in cats in Thailand. Transbound Emerg Dis. 2019; 66 (2): 948-956.
19. Alashraf, A. R., Lau, S. F., Khairani-Bejo, S., et al. First report of pathogenic *Leptospira* spp. isolated from urine and kidneys of naturally infected cats. PLoS One. 2020; 15(3): e0230048.
20. Murillo, A. Cuenca, R., Serrano, E., et al. *Leptospira* detection in cats in Spain by serology and molecular techniques. Int J Environ Res Publ Hlth. 2020; 17: 1600.
21. Spangler, D., Kish, D., Beigel, B., et al. Leptospiral shedding and seropositivity in shelter dogs in the Cumberland Gap Region of Southeastern Appalachia. PLoS One. 2020; 15 (1): e0228038.
22. Bourassi, E., Savidge, C., Foley, P., et al. Serologic and urinary survey of exposure to *Leptospira* species in a feral cat population of Prince Edward Island, Canada. J Feline Med Surg. 2021; 23 (12): 1155-1161.
23. Holzapfel, M., Taraveau, F., and Djelouadji, Z. Serological and molecular detection of pathogenic *Leptospira* in domestic and stray cats on Reunion Island, French Indies. Epidemiol Infect. 2021; 149: e229.
24. Kakita, T., Kuba, Y., Kyan, H., et al. Molecular and serological epidemiology of *Leptospira* infection in cats in Okinawa Island, Japan. Scientific Reports. 2021; 11:10365.
25. Pratt, N., Conan, A., and Rajeev, S. Leptospira Seroprevalence in Domestic Dogs and Cats on the Caribbean Island of Saint Kitts. Vet Med Int. 2017; 5904757.
26. Agunloye, C. A., and Nash, A.S. Investigation of possible leptospiral infection in cats in Scotland. J Small Anim Prac. 1996; 37(3): 126-129.
27. Millan, J., Candela, M. G., Lopez-Bao, J.V., et al. Leptospirosis in wild and domestic carnivores in natural areas in Andalusia, Spain. Vector Borne Zoonotic Dis. 2009; 9: 549-554.
28. Mylonakis, M. E., Bourtzi-Hatzopoulou, E., Koutinas, A. F., et al. Leptospiral sero-epidemiology in a feline hospital population in Greece. Vet Rec. 2005; 156 (19): 615-616.
29. Shophet, R. A serological survey of leptospirosis in cats. N Z Vet J. 1979; 27 (11): 236, 245-246.
30. Sonja, O., Sonja, R., Nataša, S., et al. Seroprevalence of Cat Leptospirosis in Belgrade, Serbia. Acta Veterinarian. 2014; 64 (4): 510-518.
31. Denipitiya, D. T. H., Chandrasekharan, N. V., Abeyewickreme, W., et al. Identification of cattle, buffaloes and rodents as reservoir animals of *Leptospira* in the District of Gampaha, Sri Lanka. BMC Res. 2017; 10: 134.
32. Carlos, E. R., Kundin, W. D., Watten, R. H., et al. Leptospirosis in the Philippines: Feline studies. Am J Vet Res. 1971; 32: 1455- 1456.
33. Mason, R. W., King, S. J., and McLachlan, N. M., Suspected leptospirosis in two cats. Aust Vet J. 1972; 48: 622-623.
34. Weis, S., Rettinger, A., Bergmann, M., et al. Detection of Leptospira DNA in urine and presence of specific antibodies in outdoor cats in Germany. J Feline Med Surg. 2017; 19 (4): 470-476.
35. Larsson, C. E., Santa Rosa, C. A., Hagiwara, M. K., et al. Prevalence of feline leptospirosis: serologic survey and attempts of isolation and demonstration of the agent. Int J Zoonoses. 1984; 11 (2): 161-169.
36. Mosallanejad, B., Ghorbanpoor Najafabadi, M., Avizeh, R., et al. A serological survey of leptospiral infection of cats in Ahvaz, south western of Iran. Int J Vet Res. 2011; 5 : 49-52.
37. Brasil de Lima, A. W., Parantoni, R. N., Feitosa, T. F., et al. Anti-Leptospira spp. antibodies in cats from the semiarid of the Paraíba State. Semina: Ciências Agrárias. 2014 ; 35 (6): 3215-3220.
38. Higgins, R., and Cayouette, P. Serological diagnosis of leptospirosis in the Province of Quebec. Can Vet J. 1978; 19: 13-16.
39. Everard, C.O., Cazabon, E. P., Dreesen, D. W., and Sulzer, C. R. Leptospirosis in dogs and cats on the Island of Trinidad: West Indies Int J Zoonoses. 1979; 6 (1): 33-40.
40. Dickeson, D., and Love, D. N. A. serologic survey of dogs, cats and horses in south-eastern Australia for leptospiral antibodies. Aust Vet J. 1993; 70 (10): 389-390.
41. Natarajaseenivasan, K., Boopalan, M., Selvanayaki, K., et al. Leptospirosis among rice mill workers of Salem, South India. Jpn J Infect Dis. 2002; 55(5): 170-173.
42. André-Fontaine, G. Canine leptospirosis - do we have a problem? Vet Microbiol. 2006; 117 (1): 19-24.
43. Akuzawa, M., Maruyama, T., Endo, Y., et al. Survey of *Leptospira* infection in domestic cats in the southern Kyushu District. Jpn Med Assoc J. 2006; 59: 45-48.
44. Jamshidi S., Akhavizadegan M., Maazi N., Ali A. G., and Bokaie, S. Serologic study of feline leptospirosis

in Tehran. Iran J Microbiol. 2009; 1: 32-36.
45. Parreira, I., Jayme, V., Walburga, E., Guimaráes, L., et al. Epidemiological features of infection through *Leptospira* spp in domestic cats (*Felis catus*) apparently healthy within the metropolitan area of Goiania, Brazil. Enciclopédia Biosfera. 2010; 6: 1-5.
46. Felt, S. A., Wasfy, M. O., El-Tras, W. F., et al. Cross-species surveillance of Leptospira in domestic and peri-domestic animals in Mahalla City, Gharbeya Governorate, Egypt. Am J Trop Med Hyg. 2011; 84 (3): 420-425.
47. Markovich, J. E., Ross, L., and McCobb E. The prevalence of leptospiral antibodies in free roaming cats in Worcester County, Massachusetts. J Vet Intern Med. 2012; 26: 688-689.
48. Desvars, A., Naze, F., Benneveau, A., et al. Endemicity of leptospirosis in domestic and wild animal species from Reunion Island (Indian Ocean). Epidemiol Infect. 2013; 141 (6): 1154-1165
49. Lapointe, C., Plamondon, I., and Dunn, M. Feline leptospirosis serosurvey from a Quebec referral hospital. La Rev Vet Can. 2013; 54 (5): 497-499.
50. Chan, K. W., Hsu, Y. H., Hu, W. L., et al. Serological and PCR detection of feline *Leptospira* in southern Taiwan. Vector Borne Zoonotic Dis. 2014; 4 (2): 118-123.
51. Talebkhan Garoussi, M., Mehravaran, M., Abdollah-pour, G., et al. Seroprevalence of leptospiral infection in feline population in urban and dairy cattle herds in Mashhad, Iran. Vet Res Forum. 2015; 6 (4): 301-304.
52. Dos Santos, L. F., Guimarães, M. F., de Souza, G.O., et al. Sero-epidemiological survey on *Leptospira* spp infection in wild and domestic mammals in two distinct areas of the semi-arid region of northeastern Brazil. Trop Anim Health Prod. 2017; 49 (8): 1715-1722.
53. Ortega-Pacheco, A, Guzmán-Marín, E, Acosta-Viana, K. Y., et al. Serological survey of *Leptospira interrogans, Toxoplasma gondii* and *Trypanosoma cruzi* in free roaming domestic dogs and cats from a marginated rural area of Yucatan Mexico. Vet Med Sci. 2017; 3(1): 40-47.

Table 4: Leptospirosis infection rates and commonly reported serovars in cats from studies conducted worldwide

Region (country)	Year of the study	Way of life	Clinical status	Number of Sera collected	Positive results	Prevalence (%)	Dominated Serovars/‖Serogroups	Cut-off value	References
Quebec (Canada)	1974-1976	NI	Suspected of having leptospirosis	19	0	0	Nd	1: 100	38
Trinidad and Tobago	NI	NI	NI	40	5	12.5	‖Canicola, Icterohaemorrhagiae, Hebdomadis	-	39
New Zealand	NI	House	NI	225	20	8.8	Hardjo, Pomona, Ballum, Copenhageni, Balanica, Canicola	1:24	29
São Paulo (Brazil)	NI	NI	NI	172	22	12.8	Pomona	1:100	35
South East Australia	1988-1990	NI	NI	59	10	16.9	Pomona, Copenhageni, Grippotyphosa, Tarassovi	1:50	40
Glasgow (Scotland)	NI	NI	Ill, leptospirosis was not suspected	87	8	9.2	Hardjo, Icterohaemorrhagiae Autumnalis	1:30	26
South India	2000	Living on the premises of rice mill	NI	9	6	66.6	‖Autumnalis, Canicola, Icterohaemorrhagiae	1:80	41
Thessaloniki (Greece)	1997-1998	Owned	Total	99	33	33.3	Rachmati, Bratislava, Ballum, Bataviae, Canicola, Panama, Pyrogenes	1:50	28
			Ill	51	18	35.3			
			Healthy	48	15	31.3			
France	NI	NI	Ill	98	47	48.0	‖Canicola, Sejroë, Australis, Icterohaemorrhagiae	-	42
Southern Kyushu (Japan)	NI	NI	NI	117	9	7.7	Autumnalis, Hebdomadis, Australis, Icterohaemorrhagiae, Pyrogenes	1:50	43
Tehran (Iran)	2003	Total	NI	111	30	27.0	Canicola, Hardjo, Icterohaemorrhagiae	1:100	44
		Stray		89	19	21.3			
		Household		22	11	50.0			

Andalucia (Spain)	2004-2007	Feral	NI	53	7	14.0	Icterohaemorrhagiae, Ballum	1:100	27
Goiânia (Brazil)	2008	NI	Healthy	330	23	6.9	Cynopteri, Djasiman, Butembo, Castellonis, Patoc	1:100	45
Mahalla (Egypt)	2006-2007	Feral	NI	2	1	100.0	Grippotyphosa	1:50	46
Ahvaz (Iran)	2007-2008	Stray	NI	102	5	4.9	Ballum, Australis	1:100	36
United States	2010	Feral	NI	63	3	4.8	Autumnalis, Pomona, Icterohaemorrhagiae, Bratislava	1:100	47
Reunion Island	2009	Stray	NI	30	8	26.6	‖Panama	1:100	48
Quebec (Canada)	2007	NI	Presented for different clinical signs	40	10	25.0	Bratislava, Autumnalis	1:100	49
Southern Chile	2011-2012	Urbanand rural cats	NI	124	10	8.1	Autumnalis, Canicola, Bataviae	1:100	6
Paraíba (Brazil)	2011	Total	Healthy	129	7	5.43	Pomona	1:100	37
		Owned		61	4	6.56			
		Stray		68	3	4.41			
Southern Thailand	2010-2011	Total	NI	225	21	9.3	Shermani, Javanica, Icterohaemorrhagiae, Australie, Pyogenes	1:100	50
		Stray cats		155	17	11.0			
		Household cats		70	4	5.7			
Quebec (Canada)	2010-2012	NI	Healthy cats	125	9	7.2	Pomona, Bratislava, Grippotyphosa Icterohaemorrhagiae	1:100	7
			Cats with kidney disease	114	17	14.9			
Belgrade (Serbia)	2012-2013	Healthy	Stray	161	45	26.7	Grippotyphosa, Icterohaemorrhagiae, Pomona, Canicola, Batavie, Australis	1:100	30
Mashhad (Iran)	2008-2010	Total	Healthy	147	10	6.8	Hardjo, Pomona, Icterohaemorrhagiae	1:100	51
		Households		42	0	0			
		Stray		52	1	0.52			
		Rural		53	9	4.77			

Northeastern Brazil	2013-2015	Rural	NI	43	2	4.7	Andamana, Patoc	1:100	52
Merida (Mexico)	2005	Owned free roaming cats	NI	13	3	23.2	Canicola, Australis	1:100	53
Saint-Christophe Island	2014-2015	NI	NI	50	2	4.0	Cynopteri, Pomona	1:100	25
Munich (Germany)	2013-2015	Outdoor	Various clinical signs	195	35	17.9	Australis, Bratislava, Grippotyphosa, Copenhageni	1:100	34
Iowa (USA)	2015-2016	Stray and shelter	NI	139	12	8.6	Pomona, Icterohaemorrhagiae, Bratislava, Hardjo, Grippotyphosa	1:100	17
Thailand	2016-2017	NI	Healthy except three	260	14	5.4	Anhoa, Autumnalis, Celledoni, Copenhageni, Djasiman, Icterohaemorrhagiae, Patoc	1:20	18
Malaysia	2017-2018	Shelter	Healthy	82	21	25.6	‖Bataviae, Javanica, Ballum	1:100	19
Spain	2017-2018	Stray and shelter	NI	244	10	4.1	Cynopteri, Ballum, Bratislava, Grippotyphosa, Proechimys	1:20	20
Appalachia (USA)	2017-2018	Shelter	NI	43	0	0	--	1:100	21
Prince Edward Island, Canada	2017-2018	Feral cats	Healthy (presented for sterilization)	20	200	10.0	Icterohaemorrhagiae, Canicola, Grippotyphosa, Bratislava, Pomona	1:50	22
Northern and western Reunion Island	2013	Domestic cats	Healthy	50	21	42.0	Icterohaemorrhagiae, Ballum (Castellonis)	1:40	23
		Stray cats (Urban)		42	13	31.0			
Okinawa Island, Japan	2016-2018	Stray	-	241	40	16.6	‖Javanica, Hebdomadis	1:80	24

NI= not identified, not cited‖= Serogroup