Sero-survey of leptospirosis in cats

Afr. J. Clin. Exp. Microbiol. 2022; 23 (4): 416-425

Zaidi et al. Afr. J. Clin. Exper. Microbiol. 2022; 23 (4): 416 - 425

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X
AJCEM/2203. https://www.aiol.info/index.php/ajcem

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Original article

Serological study of leptospirosis in cats from Algeria

*1Zaidi, S., 2Amara Korba, A., 3Bessas, A., 2Bouzenad A., 2Hamnoune, N. K., 4,5Hezil, Dj., and 6Bitam, I.

1Higher National Veterinary School, El Ali, Oued Smar, 1615, Algiers, Algeria
2Leptospira unit, Pasteur Institute of Algeria, Rue 1 of Doctor Laveran, Hamma Anassers Algiers, Algeria
3Department of Biology, Faculty of Sciences, University of Algiers 1 (Benyoucef Benkhedda University), Algiers, Algeria
4Research Laboratory Management of Local Animal Resources, Higher National Veterinary School of Algeria, ENSV, Algeria
5Department of Biology, Faculty of Sciences, M’Hamed Bougara University, Boumerdes, Algeria
6Higher School of Food Sciences and Agri-Food Industries, Algiers, Algeria

*Correspondence to: zaidi.env@gmail.com; s_zaidi@ensv.dz

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, Copenhagani, 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

Received Jun 30, 2022; Revised Jul 7, 2022; Accepted Jul 15, 2022

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Etude sérologique de la leptospirose chez les chats d’Algérie

*1Zaidi, S., 2Amara Korba, A., 3Bessas, A., 2Bouzenad A., 2Hamnoune, N. K., 4,5Hezil, Dj., et 6Bitam, I.

1Ecole Nationale Supérieure Vétérinaire, El Ali, Oued Smar, 1615, Alger, Algérie
2Unité de Leptospira, Institut Pasteur d’Algérie, Rue 1 du Docteur Laveran, Hamma Anassers Alger, Algérie
3Département de Biologie, Faculté des Sciences, Université Alger 1 (Université Benyoucef Benkhedda), Alger, Algérie
4Laboratoire de Recherche Gestion des Ressources Animales Locales, Ecole Nationale Supérieure Vétérinaire d’Algérie, ENSV, Algérie
5Département de Biologie, Faculté des Sciences, Université M’Hamed Bougara, Boumerdes, Algérie

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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 Leptospira in cats (10). According to Hartmann et al., (11), renal carriage and leptospirouria 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 - Hygiène 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 | Verdon        |
| Autumnalis             | Autumnalis       | Akiyami A       |
| Grippotyphosa          | Grippotyphosa    | Moskvka 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 (χ²) 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 information 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        | 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          | 0.2586* |
|                       | Male          | 90                     | 7 (7.8)                              | (0.5344-37.387) |         |
| Age (years)           | < 1 year      | 32                     | 0                                    | 0.1891        | 0.1996* |
|                       | > 1 year      | 112                    | 8 (7.1)                              | (0.01062-3.369) |         |
| 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 serovars, 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 Icterhemorrhagiae (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 Icterhemorrhagiae, 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 Leptospira 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.

Acknowledgments:

A special thanks to Amara Korba Anissa for her close and precious collaboration. The authors would like to thank Mr. Schiff Lyês for his precious help. Our appreciations to veterinarians of HURBAL (HUPE) for their provision of samples.

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; HDJ was involved in the methodology and manuscript writing; and BI was involved in resources provision, supervision and visualization.

Source of funding:

No funding was received for the study.

Conflict of interest:

Authors declare no conflict of interest.

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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        |            |
| 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, Balum, Copenhageni, Balanica, Canicola                                            | 1:24         |            |
| 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         |
|                           |                   |             | III                                 | 51                        | 18               | 35.3           | Canicola, Sejroe, Australis, Icterohaemorrhagiae                                                 | -            | 42         |
|                           |                   |             | Healthy                             | 48                        | 15               | 31.3           |                                                                                             |              |            |
| France                    | NI                |           | III                                 | 98                        | 47               | 48.0           |                                                                                             |              |            |
| Southern Kyushu (Japan)   | NI                |           | II                                  | 117                       | 9                | 7.7            | Autumnalis, Hebdomadis, Australis, Icterohaemorrhagiae, Pyrogenes                              | 1:50         | 43         |
| Tehran (Iran)             | 2003              | NI          | Total                               | 111                       | 30               | 27.0           | Canicola, Hardjo, Icterohaemorrhagiae                                                           | 1:100        | 44         |
|                           |                   |             | Stray                               | 89                        | 19               | 21.3           |                                                                                             |              |            |
|                           |                   |             | Household                           | 22                        | 11               | 50.0           |                                                                                             |              |            |
| Location             | Year       | Type      | Control | Seropositive | Antibodies Tested | Titers | Positive Rate |
|----------------------|------------|-----------|---------|--------------|-------------------|--------|---------------|
| Andalucia (Spain)    | 2004-2007  | Feral     | NI      | 53           | 7                 | 14.0   |               |
| Goiânia (Brazil)     | 2008       | NI        | Healthy | 330          | 23                | 6.9    |               |
| Mahalla (Egypt)      | 2006-2007  | Feral     | NI      | 2            | 1                 | 100.0  |               |
| Ahvaz (Iran)         | 2007-2008  | Stray     | NI      | 102          | 5                 | 4.9    |               |
| United States        | 2010       | Feral     | NI      | 63           | 3                 | 4.8    |               |
| Reunion Island       | 2009       | Stray     | NI      | 30           | 8                 | 26.6   |               |
| Quebec (Canada)      | 2007       | NI        | Presented for different clinical signs | 40 | 10 | 25.0 | Bratislava, Autumnalis | 1:100 | 49 |
| Southern Chile       | 2011-2012  | Urban and rural cats | NI | 124 | 10 | 8.1 | Autumnalis, Canicola, Bataviae | 1:100 | 6 |
| Paralba (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 | Shermanni, Javanica, Icterohaemorrhagiae, Austral, 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 |        |        |      |
| Location                          | Year         | Setting                  | NI | Positive | ASS | Serogroups                                                                 | Reference |
|----------------------------------|--------------|--------------------------|----|----------|-----|----------------------------------------------------------------------------|-----------|
| 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, Celedoni, 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 |
| Okinawa Island, Japan            | 2016-2018    | Stray                    | -                       | 241 | 40 | 16.6 Javanica, Hebdomadis                                                   | 1:80 | 24 |

NI = not identified, not cited  
ASS = Arthron conjugation units 

\* NI = not identified, not cited  
\* Serogroup