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Serologic study of feline leptospirosis in Tehran, Iran

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ABSTRACT

Background and Objectives: Leptospirosis is considered to be the most widespread zoonotic disease in the world and can infect a wide range of animals. Although the prevalence of clinical leptospirosis in cats is low, they are probably exposed to leptospires excreted by wild life, rodents etc. This study was performed to determine the serologic reaction of cats to leptospires and their importance in transmission of this zoonotic disease in Tehran.

Materials and Methods: Serum samples were collected from 111 stray and household cats and were tested for the presence of antibodies against leptospirosis by Microscopic Agglutination Test (MAT).

Results: Thirty (27 percent-19 stray and 11 household) of the 111 cats reacted with the various leptospiral serotypes. The dilutions of sera with positive results ranged from 1/100 to 1/600. Serologic reaction was more prevalent in domestic cats (p=0.0067). In stray cats, 18 cases were positive against *L. interrogans* serovar Canicola (94.7 percent) and one (5.3 percent) against *L. interrogans* serovar Pomona. In the household group, 6 cats (54.5 percent) reacted with L. borgpetersenii serovar Hardjo, 3 cats (27.3 percent) with *L. interrogans* serovar Icterohaemorrhagiae, one (9 percent) with *L. interrogans* serovar Grippotyphosa and one with *L. interrogans* serovar Canicola.

Conclusion: Cats can be exposed to leptospires and in optimal conditions they can infect the environment or transmit the disease to contact people.

Keywords: leptospirosis, cats, serology, microscopic agglutination test.

INTRODUCTION

Leptospirosis is an acute febrile zoonotic disease affecting the kidneys and the liver and it is of worldwide significance in many animals. It is caused by infection with antigenically distinct serovars of the parasitic species *Leptospira interrogans sensu lato*, of which at least eight have clinical significance for dogs and cats. Serovars are maintained in nature by numerous sub-clinically infected wild and domestic animal reservoir hosts that serve as a potential source of infection and illness for humans and other incidental animal hosts. Leptospires are transmitted between animals by direct or indirect contact. Direct transmission occurs through contact with infected urine, venereal and placental secretions, bite wounds or ingestion of infected tissues . Direct spread of the infection is enhanced by crowding of animals. Indirect transmission occurs through exposure of susceptible animals to contaminated water sources, soil, food or bedding. (1)

Although the prevalence of clinical leptospirosis in cats is low, they probably are exposed to leptospires excreted by wild life. Outdoor cats have the highest seroprevalence and it has been shown that household cats are more protected against leptospirosis (2). There is evidence showing that leptospiral infection can become severe clinically or even progress to death aside from the subclinical infection which is of greatest importance in spread of the infection (3). There are several serotypes

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of leptospiral spirochetes and serovars Canicola, Gripotyphosa and Pomona have been isolated from cats. Cats may also be exposed to the urine of cohabitating dogs and transmission from rodents carrying serovars Ballum or Icterohaemorrahagiae is suspected (1). Experimentally, the pathogenesis of feline leptospirosis is similar to that of the canine infection, but clinical signs rarely develop in cats despite the development of histological lesions in the kidneys and liver. It is important to be aware, however, that cats can excrete potential zoonotic leptospires in their urine for up to 3 months following experimental infection (4). A case of feline stillbirth associated with *Salmonella typhimorium* and the presence of leptospiral antigen has been reported (5).

Despite the high hygienic importance of leptospirosis in human populations, the serologic prevalence of leptospirosis in cats in Iran has not been studied yet. Thus the present serologic study was conducted in Tehran.

MATERIAL AND METHODS

Animals. Sample size was calculated based on serologic prevalence of 30% in stray cats and 20% in household cats, confidence level of 95%, precision of 10% and 10% of attrition. So, one hundred and eleven cats including two groups; stray and household were sampled in January to July 2003. Stray cats (89 cases) were collected from different parts of Tehran, Iran and 22 household cats were selected randomly from cats that were referred to the Small Animal Hospital of the Veterinary Faculty of Tehran University for routine clinical examinations. Records of age, sex and residential address (for household cats), were obtained.

Blood collection and processing. After clinical examination, blood samples were collected by venopuncture in tubes containing anti-coagulants. Collected sera were stored at -20^{o}C until required.

Microscopic Agglutination Test (MAT). Microscopic agglutination test, direct and indirect fluorescent antibody test were used as the primary screening tools, however, they are also used as diagnostic tests because there are difficulties in performing the isolation and culturing of the organism which is the initial diagnostic approaches. In this study microscopic agglutination test was used, since it has been considered as the gold standard method in the screening of leptospirosis in literature and it is the most common method.

The 111 sera were tested for the presence of antibodies against leptospirosis by Microscopic Agglutination Test (MAT) in Razi Vaccine and Serum Research Institute, Karaj, Iran. The MAT was done in two stages: an initial screening test and a final titration. At the first step, the serum samples were tested using a battery of 16 *Leptospira* antigens including Australis, Autumnalis, Ballum, Bulembo, Canicola, Cynopteri, Grippotyphosa, Hebdomadis, Icteroheamorrhagiae, Javanica, Panama, Pomona, Pyrogens, Harjo, Tarassovi, Sejroe. Dilutions of 1/50 prepared from the sera and an equal volume of antigen added to them until the final dilutions reached 1/100. Micro plates containing serum and antigens were incubated at 30^{o}C for 1.5-4 hours. To assess the rate of agglutination, micro plates of the serum-antigen mixture were transferred to 0.1 mm thick slides and viewed under a dark ground microscope at 100× magnification. Positive reactions were considered when at least 50 percent of the leptospires agglutinated. In the next step all positive sera were subjected to an endpoint titration using split sera dilutions of 1/50, 1/100, l/200, 1/400, 1/800 and 1/1600. In this investigation, a titer of 1/100 and above was considered positive.

Statistical analysis. Chi-square test was used to statistically compare the stray and household cats as well as sex-matched comparison of the prevalence.

RESULTS

Thirty (27 percent- 19 stray and 11 household) of the 111 cats reacted with the various leptospiral serotypes. The dilutions of sera with positive results ranged from l/100 to 1/600. Statistical analysis showed significant difference between stray and household cats ($p = 0.0067$).

From stray cats, 18 cases were positive against *L. interrogans* serovar Canicola (94.7 percent) and one (5.3 percent) against *L. interrogans* serovar Pomona. In the household group, 6 cats (54.5 percent) reacted with *L. borgpetersenii* serovar Hardjo, 3 cats (27.3 percent) with *L. interrogans* serovar Icterohaemorrhagiae, one (9 percent) with *L.*

Table 1. Prevalence of various serotypes of leptospires based on sex.

Serovar Group	Sex	Canicola	Pomona	Gripotyphosa	Hardjo	Icteroheamorrhagiae	Total
Household	Male	1	-	-	4		7
	Female	-	-	1	2	1	4
	Total	1	-	1	6	3	11
Stray	Male	10	1	-	-	-	11
	Female	8	-	-	-	-	8
	Total	18	1	-	-	-	19
Total	Male	11	1	-	4	2	18
	Female	8	-	1	2	1	12
	Total	19	1	1	6	3	30

Table 2. MAT results regarding the age and sex of the household cats.

Age Sex	MAT	⩽1 year	2 years	3 years	4 years	5 years	Total
Male	Positive	3	1	2	-	1	7
	Negative	2	3	1	2	1	9
	Total	5	4	3	2	2	16
Female	Positive	-	1	-	2	1	4
	Negative	1	-	1	-	-	2
	Total	1	1	1	2	1	6
Total	Positive	3	2	2	2	2	11
	Negative	3	3	2	2	1	11
	Total	6	5	4	4	3	22

Table 3. MAT results regarding the age and sex of the stray cats.

Age Sex	MAT	⩽1 year	2 years	years 3	4 years	5 years	Total
Male	Positive	6	2	1	1	1	11
	Negative	7	8	13	5	6	39
	Total	13	10	14	6	7	50
Female	Positive	3	1	1	1	2	8
	Negative	13	4	4	7	3	31
	Total	16	5	5	8	5	39
Total	Positive	9	3	2	2	3	19
	Negative	20	12	17	12	9	70
	Total	29	15	19	14	12	89

interrogans serovar Grippotyphosa and one with *L. interrogans* serovar Canicola (Table 1). MAT results regarding the age and sex of the animals are presented in the Tables 2 and 3.

Statistical analysis with chi-square test showed no significant difference based on sex in both groups (P=0.9437). However, there was a tendency to have a higher prevalence in male group (Frequency of positive reactions = 60 %).

DISCUSSION

This study showed a considerable prevalence (27%) of antibodies against leptospiral serovars in dilutions of 1/100 and above in both household and stray cats. However, in other studies, more diluted sera (such as 1/30 and 1/50) were considered positive (6). According to these results, the presence of positive test results in dilutions of 1/100 and above would be an indicator for active infection, previous infection or vaccination (7). Since cats were not routinely vaccinated against leptospirosis and they are innately resistant to the clinical form of the disease, positive test results probably would be indicative of their exposure to the leptospires. Contact with rodents, wild life as well as stray dogs may be considered as the source of this exposure (1).

A higher serologic prevalence in household cats (50%) compared to stray cats (21.3%) has been shown in this study. This can be due to the fact that household cats in Iran like stray ones can routinely be out of the house and they are at the risk of exposure to the environmental infections. Therefore, limiting the household cats to the house would play a protective role against infection, which confirms the observation of Childs et al. (2). The male versus female ratio, 1.5:1, was expected according to the results of the studies of leptospirosis in the United States (8).

Close contact between stray cats and dogs may be the reason for high positive sera tests with *L. interrogans* serovar Canicola in this population whereas the reason for high seropositivity with *L. borgpetersenii* serovar Hardjo in household cats is not clear.

There are a few studies about leptospirosis in cats that show different seroprevalence rates and different serotypes. In one study, 30.4 percent of cats were seropositive with titers of 1/100-1/200. In that study, 16 cats were seropositive to *L. interrogans* serovar Bataviae, 14 to *L. interrogans* serovar Pomona, 12 to *L. interrogans* serovar Sejore, 2 to *L. interrogans* serovar Grippotyphosa, *L. interrogans* serovar Austtratis, *L. interrogans* serovar Icterohaemorrhagia and 16 to two or more serovars (9). Another study showed that, 5.6 percent of 142 cats were seropositive (10). An investigation in Scotland that was performed on 87 cats from the Glasgow area showed that 8 cats (9.2 percent) reacted serologically with antigens of three serovars of leptospires. Five of them were seropositive to *L. borgpetersenii* serovar Hardjo, 2 to *L. interrogans* serovar Automnalis and one cat to *L. interrogans* serovar Icterohaemorrhagiae (11). A serologic survey of cats in Australia showed that 16.9 percent of sera had serologic evidence of past exposure to leptospirosis (12). The same investigation in Brazil showed 33.3 percent positive results (13).

According to these articles, cats can be exposed to the leptospires and in optimal conditions may spread these organisms in the environment. The 27 percent positive sera tests in Tehran, shows that cats can be exposed to the organism and may have significant role in the spread of the disease. The protective role of vaccination in releasing the organism is not clear and further investigation is needed.

Reviewing the serotypes with positive reactions showed L. *interrogans* serovar Conicula is the most common serotype in stray cats and *L. borgpetersenii* serovar Hardjo in households. Further studies using isolation and culture of the bacteria, ELISA and PCR need to be done in order to obtain more information about different serovars infecting cats, their reservoirs in the environment and the role of the cat in transmitting the infection.

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