A serological survey of Leptospiral infection of cats in Ahvaz, south-western of Iran

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Key Words:
Leptospirosis; seroprevalence; cat; zoonosis; Ahvaz; Iran.

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Abstract

Leptospirosis is a zoonosis with numerous reservoir hosts. The disease is caused by infection with various serovars of *Leptospira interrogans sensu lato*. This study was conducted to evaluate the seroprevalence of leptospiral infection in stray cats in Ahvaz (south-western Iran) from April 2007 to June 2008. Blood samples were collected from 102 stray cats and screened for leptospiral infection using the microscopic agglutination test (MAT). Five of the 102 cats (4.9%) were serologically positive for at least one serovar of *L. interrogans*. The greatest number of reactors was for *L. interrogans* serovar balum (five serum samples). Antibodies against more than one serovar (namely, serovars balum and australis) were detected in one sample. All positive titers were detected at 1:100 dilution. The prevalence of leptospiral infection was 5.3% and 4.4% in male and female cats, respectively. There was no significant difference in positive titer prevalence between different sexes but prevalence was significantly different between age groups (P = 0.021), as all cats with positive titers were three years of age or more. This is also the first report of infection with *L. interrogans* serovars balum and australis from cats in Iran.

Introduction

Leptospirosis is an infectious disease of worldwide significance that affects animals and humans and is caused by *Leptospira interrogans sensu lato*. Despite the presence of leptospiral antibody titers in feline populations, clinical reports of leptospirosis in cats are infrequent (Greene *et al.,* 2006). Although cats seroconvert after exposure to leptospires, they appear to be less susceptible than dogs to both spontaneous and experimental infections (Tilley *et al.,* 2000; Greene *et al.,* 2006). The serovars canicola, grippotyphosa and pomona have been isolated from cats. Cats may be exposed to infected urine of cohabiting dogs but transmission is also suspected to result from contact with rodents that may carry the balum or icterohemorrhagiae serovars. Leptospira can infect people through contact with an environment contaminated by the urine of a shedder host, such as rodents. Most animals remain carriers long after initial infection and continue to excrete bacteria into water sources and soil. *L. interrogans* can penetrate into the body of another host through cuts in the skin (Greene *et al.,* 2006).

Long-term survival of pathogenic leptospires outside the host requires a warm and moist environment with near-neutral pH. Diagnosis of leptospirosis is often made by serological testing because culture is expensive. A variety of serological tests have been developed and these show varying degrees of serogroup and serovar specificity (Tilley *et al.,* 2000; Hartmann *et al.,* 2005; Greene *et al.,* 2006). Two tests have a role in veterinary diagnosis, namely the microscopic agglutination test (MAT) and enzyme-linked immunosorbent assay (ELISA). MAT is sensitive and specific and it is considered to be the standard serological test for the diagnosis of leptospirosis.

Feline leptospirosis was first reported in Iran in the Tehran cat population (Jamshidi *et al*., 2009). Khorami *et al.* (2009) reported that urinalysis or dipstick was not suitable for screening dogs that are actively shedding leptospires in their urine. Serosurvey has generally shown exposure rates of 10% or less in cats (Greene *et al.,* 2006). The aim of this survey was to determine the seroprevalence of leptospiral infection in stray cats in Ahvaz, south-western Iran.

Materials and Methods

Between April 2007 to June 2008, blood samples were collected from 102 stray cats in different areas of

Ahvaz. According to the dental formula, the cats were divided into two age groups, specifically less than or more than 3-years-old. Of the cat breeds, 99 were DSH (domestic short hair) and three were DLH (domestic long hair). At the time of blood collection, all animals appeared healthy and showed no clinical signs suggestive of leptospirosis. From the jugular vein of each cat was collected 2 ml of blood. Before blood collection, cats were sedated by injection with ketamine (10 mg/kg) and acepromazine (0.15 mg/kg). Using the MAT, sera were tested for antibodies against seven live antigens of *Leptospira interrogans* (serovars pomona, canicola, hardjo, balum, icterohaemorrhagiae, grippotyphosa and australis). The tests were performed in the Leptospiral Research Laboratory (Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran) mainly as described by Turner "MAT method" with some modifications (National Veterinary Services Laboratories, 1987).

All serum samples were two-fold serially diluted in phosphate buffer solution (PBS) in a microtiter plate (Greiner) up to 1:800 dilutions but starting with an initial 1:50 dilution. Then, 10 µL of serum dilution was added to 10 µl of the appropriate antigen on a microscopic slide. This was placed in a plate with moist paper to avoid evaporation and incubated at 30°C for 90 min. Finally, the slide was examined microscopically under dark-field conditions (Olympus BX50). One antigen control and two (positive and negative) standard serum controls were used for each assay. Titers of ⩾ 1:100 were considered positive. The endpoint titer was determined as the greatest serum dilution showing agglutination of at least 50% of the leptospires (Hajikolaie *et al*., 2005). To examine whether there were any statistically significant relationships between the prevalence of positive cases and other factors such as cat age and sex, data were examined using Fisher's exact test with a confidence interval of 95%.

Results

Five of the 102 cats (4.9%) were serologically positive for at least one serovar of leptospira. The greatest number of reactors was for *L. interrogans* serovar balum (five serum samples) followed by the australis serovar (one sample). Antibodies against more than one serovar (namely, serovars balum and australis) were detected in one sample. The prevalence of leptospiral infection was greater in male (5.3%) than female (4.4%) cats but this was not statistically significant (P = 0.61). All cats with positive titers were 3-years-old or more meaning that significantly more positive titers were observed in this age group compared with the group of cats less than 3-years-old (P = 0.021). Distribution of leptospiral infection was not significantly different across various areas of Ahvaz. All of the cats with positive titer were DSH but a comparison of the prevalence between DSH and DLH breeds was not performed because 99 of the 102 cats were DSH and only three cats were DLH. The results are summarized in Tables 1 and 2.

Table 1: Status of leptospiral infection (2007-2008) by MAT according to different serovars of *L. interrogans* in serum samples from stray cats in Ahvaz, south-western Iran.

Serovars	Number	Percent
Balum	4	80%
Balum + australis	1	20%
Total	5	100%

Table 2: Status of leptospiral infection (2007-2008) by MAT according to age and sex of stray cats in Ahvaz, south-western Iran.

	< 3 years		> 3 years	
Sex	Neg.	Pos.	Neg.	Pos.
Male	25	0	29	3
Female	29	0	14	2
Total = 102	54	0	43	5

Discussion

The results from this survey showed that 4.9% of the cats were positive for *L. interrogans* serovars balum and australis. The present study is the first serological survey to determine the predominant serovars of *L. interrogans* in the feline population of Ahvaz, Iran. Based on serological testing, the prevalence of leptospiral infection in cats has been reported to be 4.5 to 14.0% in Spain (Millan *et al*., 2009[a]; Millan *et al*., 2009[b]), 48% in France (Andre-Fontaine, 2006), 66.6% in India (Natarajaseenivasan *et al.,* 2002), 9.2% in Scotland (Agunloye *et al.,* 1996), 12.5% in Trinidad (West Indies) (Everard *et al.,* 1979), 27% in Tehran (Jamshidi *et al.,* 2009), and 18.2% in Tyrol (Sebek *et al.,* 1976). These results confirm that prevalence of leptospiral infection in cats is different not only between countries but also between different areas within a country. These differences may be a consequence of environmental factors, as these can influence the development of leptospiral infection in animals and human. Significant variation is seen in the duration of survival of different *L. interrogans* serovars according to the pH of soil and water (Greene *et al.,* 2006). In the United States and Canada, a positive correlation has been reported between prevalence of leptospirosis in dogs and average rainfall (Ward *et al*., 2002).

In a survey in Tehran, Thirty (19 stray and 11 household) of the 111 cats (27%) reacted with the various leptospiral serotypes by MAT. In stray cats, 94.7% and 5.3% of these positive results were for the serovars canicola and pomona, respectively (Jamshidi *et al.,* 2009). In contrast, the prevalence of leptospiral

infection in cats in Ahvaz is relatively low (4.9%). In the present survey, the highest number of reactors was for *L. interrogans* serovar balum (in five samples), while antibodies against more than one serovar (serovars balum and australis) were found in only one sample. The cats in Ahvaz are probably exposed to leptospires excreted by wildlife. The temperature requirement for maximal leptospiral survival may explain the difference in leptospiral prevalence in these different parts of Iran. Temperatures in Ahvaz can be up to 50 C in summer and hot weather and dry soil can decrease the survival of leptospires (Avizeh *et al.,* 2008), which may explain the lower prevalence of cases compared with Tehran. In the present study, antibodies for more than one serovar were found in only one serum. In serological tests for leptospirosis, the results often indicate infection by more than one serovar, which may be due to mixed serovar infections. Our results showed that all positive titers were at 1:100 dilution for all serovars. The prevalence of infection and titers of 1:100 reveals that leptospiral infection is relatively low in cats in Ahvaz (Greene *et al*., 2006). The results of the present study also indicate that there is no significant relationship between the sex of cats and infection. However, cats of three years of age or more were at significantly greater risk of infection than cats less than 3-years-old (P= 0.021). A possible explanation is the increasing likelihood for exposure of older cats to leptospires. Previous studies have shown that leptospiral prevalence is greater in older animals (Ward *et al*., 2004). The prevalence of *leptospirosis* in dogs in Ahvaz was reported to be 5.4% (8/149) (Avizeh *et al*., 2008), which is similar to our feline data (4.9%). The predominant titers were against the hardjo serovar of. *L. interrogans*. These results suggest that animals such as dogs and cats have reduced access to stagnant water and contaminated environments. In addition, cats are adapted to live around houses and pathogen transmission appears to be slower in this habitat. For these reasons, cats have a lower chance of being exposed to leptospires in infected water, which can infect the animal through direct contact the mucous membranes of eyes, nose, and mouth. Nevertheless, the results of the present study do not indicate the sources of infection in the cats. The higher prevalence of leptospiral infection in other animals in Ahvaz, such as cattle (53.79%), horse (27.88%), buffalo (58.73%) and donkey (40.00%) (Hajikolaei *et al* 2005), is probably due to their greater access to stagnant water and contaminated environments. These animals live in groups near water, which this can increase the likelihood of infection. Crowding of animals can also enhance spread of infection. Although serological surveys may provide an estimate for the exposure rate of cats, it does not provide information regarding how many cats are actively shedding leptospires and posing a potential zoonotic risk. Despite a low prevalence of seroreactivity, the presence of antibodies against *L. interrogans* in cats is a public health concern due to the close contact between cats and man, which provides a link between an environmental reservoir and humans. Among the two serovars that were found in our study, *L. interrogans* serovar balum was the most prevalent. Wild animals and rodents are the main reservoirs for the balum serovar in cats and this suggests that the cat population in Ahvaz may have been exposed to one of these reservoir species directly or through environmental contamination by the urine of these animals.

Following the introduction of a bivalent vaccine for the protection of cats against leptospirosis due to the canicola and icterohaemorrhagiae serovars, the worldwide incidence of disease attributed to these serovars has decreased (Greene *et al.,* 2006).

Prevention steps include vaccination of animals and keeping rodents away from the environment that animals live in. In addition, animals should be kept away from areas in which the *L. interrogans* bacteria thrive, such as stagnant water, marshes, ponds, and muddy areas. Humans should avoid contact with animal urine (Lilenbaum *et al*., 2004). Our survey provides preliminary data about leptospiral infection in cats in Ahvaz, South-western Iran. More investigations are needed to clarify the epidemiology of *leptospirosis* infections in different areas of Iran.

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