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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Received: 30 August 2010, Accepted: 27 December 2010

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 leptospiro 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. The aim of this survey was to determine the seroprevalence of leptospirosis 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, 2 ml of blood were collected. 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. Titer 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 leptospiroa. 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 leptospirosis 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 leptospirosis 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.

**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 leptospirosis infection in cats has been reported to be 4.5 to 14.0% in Spain (Millan et al., 2009; Millan et al., 2009), 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 leptospirosis 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 leptospirosis 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 leptospiroserotypes 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 leptospiroserotype canicola and pomona, respectively (Jamshidi et al., 2009). In contrast, the prevalence of leptospirosis
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 leptosporal 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 reactors were at 1:100
dilution for all serovars. The prevalence of infection
and titers of 1:100 reveals that leptosporal 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
leptosporal 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 leptospirosis infection
in cats in Ahvaz, South-western Iran. More investigations
are needed to clarify the epidemiology of *leptospirosis*
infections in different areas of Iran.

Acknowledgments

This survey was supported financially by the
Research Council of Veterinary Faculty, Shahid
Chamran University of Ahvaz.

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