



Short Communication

Antibody detection against *Leishmania infantum* in sera of companion cats in Ahvaz, south west of Iran

Mosallanejad^{*1}, B., Avizeh¹, R., Razi Jalali², M.H., Pourmehdi³, M.

1. Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

3. Department of Food hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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ABSTRACT

Leishmaniasis is an important and common zoonotic disease with a great impact on public health. In the present study, a total of 195 companion cats of different ages were examined for serum antibody detection against *Leishmania infantum* by immunochromatography assay. The cats were selected from those referring to Veterinary Hospital of Ahvaz University, south west of Iran from May 2009 to March 2012. Classification was made by age, sex, breed, region and season. The studied cats were divided based on age into three groups (<1 year, 1 – 3 years and > 3 years) and based on area into five regions (north, east, west, south and central). The results were analyzed by using Chi-square analysis, Fischer's exact test and Z test. Eighteen of 195 serum samples (9.23%) had antibody against *Leishmania infantum* (%95 Confidence Interval: 5.1-13.3%). Prevalence was significantly higher in adult cats above 3 years (28.57%; 14 out of 49) compared with mean-age cats 1- 3 years (3.57%; 3 out of 84) (Odds Ratio: 10.8) and less than 1 year (1.61%; 1 out of 62) (OR: 24.4) ($P<0.001$). Prevalence was higher in male cats (9.82%; 11 out of 112) than females (8.43%; 7 out of 83), the spring season (13.04%; 6 out of 46) and north region (13.89%; 5 out of 36), but the difference was not significant between the prevalence of infection relative to host gender, season and region ($P>0.05$). In conclusion, it is necessary to control cat's population in these area particular adult cats to reduce risk of infection transmission to other animals and human.

Keywords: *Leishmania infantum*, Cat, Prevalence, Immunochromatography assay, Ahvaz.

INTRODUCTION

Leishmania species are intracellular, protozoan organisms, transmitted by the bite of sandflies, causing leishmaniasis in human and animals. The main observed clinical forms are cutaneous and

mucocutaneous. Mediterranean visceral leishmaniasis is one of the most important and dangerous zoonotic diseases that affect people and domestic dogs. It is a severe, often fatal disease spread from dog-to-dog. Dogs are considered as the main reservoirs for *Leishmania infantum* infection and they can display illness signs too (Mohebali *et al* 2005). Information on the prevalence of Feline leishmaniasis is necessary to

*Author for correspondence.Email: bmosallanejad@scu.ac.ir

define control measures for zoonotic leishmaniasis. It has been reported sporadically in cats in various parts of the world (Slappendel & Ferrer 2006, Maroli *et al* 2007). Visceral leishmaniasis is dispersely endemic in at least five provinces of Iran including East Azerbaijan, Fars, Kohkiloyeh and Boyerahmat, Ardabil and Bushehr (Mohammadi-Ghalehbin *et al* 2011, Alborzi *et al* 2008). *Leishmania infantum* is endemic in many parts of Iraq that is adjacent to Khuzestan province (Gani *et al* 2010). Despite the fact that antibodies against *Leishmania* spp. are commonly found in dogs, there are few data available for the presence of antibodies in cats (Papadopoulou *et al* 2005). Cats may have a role as reservoir host of the parasite and as they live close together with humans and dogs, this role should be defined. In Iran, large numbers of cats are found roaming residential streets, so they can be an important potential source of transition of infection to other animals (Akhtar Danesh *et al* 2010). Leishmaniasis in cats was first described in 1912 in Algeria (Maia and Campino 2011). Several cases of both visceral and cutaneous forms have been reported in the America, Europe, Africa and Asia (Cardoso *et al* 2004, Solano-Gallego *et al* 2007). Nonetheless, the real susceptibility of cats to infection by *Leishmania* spp. and the outcome of leishmaniasis in them are poorly understood (Simoes-Mattos *et al* 2005). The clinical features associated with *Leishmania* infection are not pathognomonic and can be confused with many other cat diseases. On the other hand, cats can suffer different immunosuppressive conditions caused by viruses such as *Feline leukemia virus* (FeLV) and *Feline immunodeficiency virus* infections (FIV), which can favor multiplication of the parasite, and facilitate transmission of the agent (Slappendel and Ferrer 2006, Tilley and Smith 2005). Several laboratory methods have been developed to detect antigen or antibody in the serum of infected cats such as PCR, ELISA, DA (Direct Agglutination assays), IFAT (Indirect Fluorescent Antibody Test), Microscopy and culture methods, Western blotting and immunochromatography assay (ICA). Though these tests are more sensitive,

specific and more reproducible, but they are just expensive. ICA is one of the most common rapid field diagnostic methods used in clinical practice. Specificity and sensitivity for kits of *Leishmania infantum* Ab Test (Biotech Co., Ltd, Shanghai, China) were found to be highly 98.8% and 100% respectively (Otranto *et al* 2004). Few studies have been reported on the distribution of this disease in the Iran cat population, so the aim of the present study was to investigate the antibody detection of *Leishmania infantum* in the serum samples of companion cats in Ahvaz area, southwestern Iran.

MATERIALS AND METHODS

Study area and sample population. The present survey was performed in Ahvaz area, southwestern Iran that is located at an elevation of 12 meters above sea level and the climate is warm-humid. In this study, a total of 195 companion cats of different ages were examined for serum antibody detection against *Leishmania infantum* by immunochromatography assay. The cats used in this study were referred cases to Veterinary hospital of Ahvaz University from May 2009 to March 2012. Some of cats were kept indoors without free access to outside sources. Classification was made by age, sex, breed, region and season. Information about companion cats was taken from their owners. The studied cats were examined and the clinical trials of signs were recorded. They were divided based on age into three groups (<1 year, 1-3 years and >3 years) and based on area into five regions (north, east, west, south and central). Sixty two of the studied cats had age less than 1 year, 84 were 1-3 years and 49 had age above 3 years. Number of male and female cats was 112 and 83 respectively. Most of the studied cats (174) were domestic short hair (DSH). Number of cats that were kept in the house was important in the present study. Age was estimated by dental formulary or owner information's.

Laboratory methods. The collection of the blood samples was performed from jugular or femoral veins, with no evident clinical signs of disease and allowed to

clot and centrifuged for 5 min at 1800×g. Serum samples were stored at -70 °C and frozen sera were subsequently sent to the Department of Parasitology, for serologic testing. Antibody against *Leishmania infantum* was detected with a commercial rapid test kit (*Leishmania infantum* Ab Test, Biotech Co., Ltd, Shanghai, China) following the manufacturer's instructions. Sensitivity and specificity of these kits were 98.8% and 100% respectively (Otranto *et al* 2004). We added four drops of the serum sample into the holes using the dropper, drop by drop and slowly. As the test began to work, we saw purple color move across the result window in the center of the test device. The presence of only one band within the result window indicates a negative result. The presence of two color bands (T and C) within the result window indicates a positive result. Finally, test results were interpreted following the manufacturer's instructions (Catalog No. W81118, Shanghai, China).

Statistical analysis. Cats were grouped by age, sex, breed, season and geographic area to determine whether these factors were associated with *leishmania* infection, using chi-square analysis, Fisher's exact test, Z test (calculation of Confidence interval) and Logistic regression (calculation of Odds Ratio). Statistical comparisons were carried out using SPSS 16.0 statistical software. Differences were considered significant when P< 0.05.

RESULTS AND DISCUSSION

Eighteen of 195 serum samples (9.23%) had antibody against *Leishmania infantum* (%95 CI: 5.1-13.3%). Prevalence was significantly higher in adult cats above 3 years (28.57%; 14 out of 49) compared with mean-age cats 1- 3 years (3.57%; 3 out of 84) (OR: 10.8 and 95% CI: 2.92-39.97) and less than 1 year (1.61%; 1 out of 62) (OR: 24.4 and 95% CI: 3.08-193.54) (P<0.001). Of course the difference was not significant between cats less than 1 year compared with 1-3 years (P>0.05). Most of the studied cats were apparently healthy and were referred for vaccination, castration or ovariohysterectomy and other reasons such as viral

infections. Clinical signs were not specific for diagnosis of leishmaniasis. Prevalence was higher in male cats (9.82%; 11 out of 112) than females (8.43%; 7 out of 83), the spring season (13.04%; 6 out of 46) and north region (13.89%; 5 out of 36), but the difference was not significant between the prevalence of infection relative to host gender, season and region (P>0.05). Fifteen out of 64 (23.44%) cats were *Leishmania* positive from multi-cat households, while 3 out of 131 (2.29%) were positive among single cat households. The difference was significant between cats that lived as group and single (P<0.05). Prevalence in other seasons (winter, summer and autumn) were (7.84%; 4 out of 51, 9.38%; 6 out of 64 and 5.88%; 2 out of 34) respectively. Prevalence in other regions (east – west - south and central) were (5.26%; 2 out of 38, 10.81%; 4 out of 37, 6.67%; 3 out of 45 and 10.26%; 4 out of 39) respectively also (Table 1). One hundred seventy four out of 195 (89.23%) of companion cats were DSH breed. Fifteen out of 195 (7.69%) of the studied cats were Persian and six out of 195 (3.08%) were Domestic long hair (DLH).

Table 1. Prevalence of *Leishmania infantum* infection in companion cats of different ages and regions in Ahvaz district, Iran by ICA, 2009-2012.

Region	<u>Age</u>		<u><1 year</u>		<u>1- 3 years</u>		<u>>3 years</u>	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
North	12	1	13	1	6		3	
East	11	0	16	0	9		2	
West	13	0	13	0	7		4	
South	13	0	22	1	7		2	
Central	12	0	17	1	6		3	
Total =	61	1	81	3	35		14	
	195							

The present study that is the first report on prevalence of feline leishmaniasis in companion cats in Ahvaz district revealed that 9.23% of referred cats had antibody against *Leishmania infantum*. The reason for a low relatively seroprevalence of *Leishmania* infection in this area, may be related to short activities of sandflies, due to hot weather, nevertheless the results indicate that antibody is present in sera of companion cats of this area. Due to close contact

of cats' together, other animals and human, they can be an important potential source of transmission of infection (Slappendel and Ferrer 2006). Of course, the susceptibility of cats to infection with *Leishmania* spp. and the outcome of infection are poorly understood in cats (Simoes-Mattos *et al* 2005). Over the few years, several cases of feline leishmaniasis have been reported in Iran. In a research, forty stray cats were captured from two areas where VL is endemic, Fars and East Azerbaijan provinces. *Leishmania* parasites were detected in 4 out of 40 cats (10%). The parasite was isolated from the spleen of three and the liver of one cat. The parasites isolated from the four cats were all *Leishmania infantum* (Hatam *et al* 2010). Recently, a nested PCR has been used in Ahvaz (the capital of the province of Khuzestan) to confirm the microscopical diagnosis of CL and to identify the causative parasites. The 100 smears investigated were all found amastigote-positive by microscopy and PCR-positive for either *L. major* DNA (97 smears) or *L. tropica* DNA (three smears). The predominant species causing CL in Ahvaz was *L. major* (Ghasemian *et al* 2011). Although the cat is a rare host of *Leishmania* spp. but, it is considered to play an active role, in the epidemiology of this disease in some area. This animal is believed to have a high degree of resistance, as observed following experimental infection, which is probably dependent on genetic factors, not strictly related to cell mediated immunity (Mancianti 2004, Diakou *et al* 2009). In the present survey, prevalence was significantly higher in adult cats above 3 years compared with cats between 1-3 years and less than 1 year. The increasing prevalence of the infection with the age can be related to the time exposed to the phlebotomine activity. Minimizing exposure is the best method for prevention of infection. A higher seroprevalence was seen in male companion cats (9.82%) than females (8.43%) also. It can be explained by the territorial habits associated with

males, of course the difference was not significant. It doesn't seem sex to be a determining factor as other studies have concluded (Martin-Sanchez *et al* 2007). Bresciani *et al* (2010) found 0.7% (2/283) of cats positive for *Leishmania* spp. by the IFA method in Araçatuba, Brazil. In their study, two females were positive, a young mongrel and an adult feline. Most of the companion cats were DSH breed (89.23%) in our survey. 7.69% of the studied cats were Persian and 3.08% DLH. Prevalence was higher in the season of spring (11.32%), but the difference was not significant. The occurrence of *Leishmania* spp. in the cats of the present study was not correlated to the variables sex, breed, region and season. No statistical association was found between seroreactivity to *L. infantum* and breed, age, sex, habitat, access to outdoor environment, contact with other animals, arthropod exposure history, endoparasite treatments, travel history, clinical signs, and FeLV/FIV status (Ayllon *et al* 2008). The first case of natural infection of a domestic female cat has been reported by *Leishmania braziliensis* in French Guiana (Rougerona *et al* 2011). In the present study, the seropositive cats had not specific signs for leishmaniasis. The difference was significant between cats that lived as group and single also. 23.44% were *Leishmania* positive in multi-cat environments, while 2.29% were positive in single cat households. Extensive surveys, out of transmission season, should be performed in a representative sample using distinct methodologies to obtain an accurate prevalence. Despite this evidence, the epidemiological importance of cats in leishmaniasis is still controversial. They are often recognized as accidental hosts, and as secondary or alternative reservoir hosts for dogs. Thus, from an epidemiological and control perspective it would be very important to evaluate the proportion of transmission in endemic areas attributable to cats to clarify if these animals are reservoir hosts

sustaining (Maia *et al* 2008, Diakou 2000). Several studies have investigated the presence of leishmaniasis in domestic felids through epidemiological surveys using serological or molecular diagnosis (Dahroug *et al* 2010). From all previous studies, *Leishmania infantum* was proven to infect cats in Italy, Spain, Portugal, France, Saudi Arabia and Brazil (Morsy *et al* 1999, Pennisi *et al* 2002, Poli *et al* 2002, Mancianti 2004, Tabar *et al* 2008, Maia *et al* 2008). In Saudi Arabia, natural *Leishmania* infection in sand cats (*Felis margarita*) was investigated. Seropositivity using an IHA was 40%, microscopic examination showed Donovan-like amastigotes in 40% and 20% of splenic and liver samples, respectively (Morsy *et al* 1999). In European countries, the prevalence of leishmaniasis in domestic cats was found to range from 13% to 30.4% by PCR (Maia *et al* 2008). In an endemic area of Brazil, the prevalence of visceral leishmaniasis was 25% in domestic cats (Savani *et al* 2004). In reviews by Gradoni (1999) the results from several serological surveys in different countries are stated. In Europe, feline leishmaniasis cases have been described from Portugal, France, Spain and Italy (Hervas *et al* 1999; Mancianti 2004; Ozon *et al* 1998). In France the prevalence in cats reached 12.4% compared with the canine prevalence rates of 26.5% (Ozon *et al* 1998). In Italy the prevalence was up to 68%, depending on the area of the country (Poli *et al* 2002). In Spain, feline seroprevalence has been found up to 60% (Martin-Sanchez *et al* 2007). These results may be due to difference in the analytical procedures between laboratories, general physical and climatological characterization of areas, so that ecological conditions are suitable and ready for abundance of phlebotomine sand flies. In most reports the specific antibodies are absent or low suggesting that serology is probably not the best method of diagnosis. This low level of antibodies can be related to the fact that cats do not suffer from disequilibrium of the immune status which will lead to overproduction of

antibodies. The presence of specific antibodies only indicates that the animal was exposed to infection (Slappendel and Ferrer 2006, Todoli *et al* 2009). When the seroprevalence in cats is compared to the one in the canine population of the same area, usually cats are found to be less seropositive. In the area of the present study, the prevalence of *Leishmania* spp. antibodies in dogs was 13.8% (29 out of 210) (Avizeh *et al* 2007; Mohebali *et al* 2005), nearly 1.5 times higher than the cats. This seems to correspond well with the reports that cats are more refractory than dogs to the infection of *L. infantum* (Poli *et al* 2002). This is explained by the hypothesis that the immune response in cats, mainly cellular immunity, is effective enough to control the infection and confer a certain degree of resistance, if there are not immunosuppressive events such as FeLV or FIV (Solano-Gallego *et al* 2007). In the present study, we tested *Leishmania* spp. exposure in cats only by serology (ICA), so an underestimation of the actual rate of infection may be possible. Surveys using techniques such as PCR and cellular immunity tests should be performed in cats to estimate better any *Leishmania* spp. infection in the future. Of course, in a survey, ICA test had been showed 97% sensitivity and 100% specificity, while IFAT sensitivity was 99% and specificity was 100% (Otranto *et al* 2004). These results show that ICA is a rapid, sensitive, and specific diagnostic test particularly useful in mass-screening surveys for assessing the spread of leishmaniasis in different areas. As the clinical signs are unspecific and similar to those observed in other diseases commonly found in cats, leishmaniasis may be taken into consideration concerning the differential diagnosis and consequently, diagnostic tests should be performed in order to investigate the possibility of *Leishmania* infection (Poli *et al* 2002). As none of the positive cats are showing clinical signs, the possibilities are that they were either infected, remaining asymptomatic carriers up to the date of examination, or simply exposed to the organism by sandflies' bite and had killed off the promastigotes before an infection have developed. Whatever the case may be, the detection of antibodies

in cats in Iran questions the possibility of the epidemiological role of this animal species in canine or human leishmaniasis in the country. Despite the low seroprevalence, feline leishmaniasis is always a possibility, especially in endemic areas. Our study showed that available tests such as ICA are more valuable for clinicians who have already narrowed down the diagnosis to leishmaniasis. The lack of an effective prophylactic drug or vaccine against infection, the expense, side effects and difficulties associated with treatment of the disease, serve to emphasize the importance of vector control in disease prevention. Further studies such as molecular, microscopy and culture methods of the parasite are necessary to investigate their participation in the epidemiological chain of leishmaniosis.

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