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RESEARCH ARTICLE

SERO-PREVALENCE OF LEPTOSPIRA SPP. IN HOUSEHOLD AND STRAY CATS BY MICROSCOPIC AGGLUTINATION TEST

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ABSTRACT

Leptospirosis is a zoonotic disease of worldwide significance in human being and many animals. This zoonotic disease causes infection in cats and other pet animals and these animals can influence human's health. This study have been performed for detection of sero- epidemiology of leptospirosis and evaluation of sero- prevalence of Leptospira serovars in both household and stray cats by Microscopic Agglutination Test (M.A.T). Serum samples of 71 cats in two household and stray groups (31 household cats and 40 stray cats) were collected. Specimens were examined through M.A.T, against antigens of serovars: canicola, grippotyphosa, icterohaemorrhagiae, pomona, hardjo, autumnalis, ballum. Only one of stray cats (2.5%) showed 1:100 (or more) serum- titer against Leptospira pomuna. The antibody titer was not observed against more than one serovar in any cats. Among the household cats, 6 of them showed suspicious titer (+2), which 5 cats had titer against grippotyphosa serovar and one cat against hardjo serovar. Among the stray cats 6 of them showed suspicious titer (+2), exactly same as household group. With attention to the lack of regular and annual vaccination of household and stray cats against these organisms, the results of this assessment are accurate. Fortunately, infection percentage at Tabriz is low in contrast of other part of Iran. It seems that lack of suitable conditions and climates for growing of these organisms is the most important preventive mechanism or Leptospirosis in cats is initially rare and appears to be mild although very little is known about the disease in this species.

INTRODUCTION

Leptospirosis is a world-wide zoonotic disease. Leptospiras are aerobe spirochetes which have both gram positive and negative bacterias' specifications (Hatman *et al*., 2010). They are thin, flexible and filamentous (0.1 - 0.2 μm width and 6 – 12 μm length) which are made of hooked spirals. The genus Leptospira is an incredibly varied group of organisms, containing hundreds of serovars and genetic types (more than 250 serovars). Although the pathogenic importance of many serovars is unknown, 6 - 8 are thought to be pathogenic in dogs and probably in cats (Goldstein *et al*., 2006). These serovars can be kept by many wild and domestic animals and these hosts are as the main sources of infection for human and other animals (Green *et al*., 1990). Meanwhile, dogs are most commonly affected. Leptospirosis in cats is rare and appears to be mild although very little is known about the disease in this species.

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Leptospira interrogans serovars canicola and icterohaemorrhagiae, Leptospira kirschneri serovar grippotyphosa are the most common serovars which was isolated from dogs with Leptospirosis infection (Green*et al*., 1990). Leptospiras are going over the animals by direct transmission, venereal, embryonic, biting wounds or consumption of infected meats. Infection usually increases in crowded places. Indirect transmission occurs in contact of infected animals with contaminated vegetables, soil, food, water and other stuffs. Spirochetes are also preserved in insects and other invertebrate hosts; but roles of this kind of transmitters in spreading these organisms are still unknown (Green *et al*. 1990). Cats infected with leptospirosis have clinical signs that are usually mild or inapparent, although there are leptospires present within the blood and urine of the animal as well as histological evidence of liver and kidney inflammation (Greene *et al*. 1998). Animals which cured from acute infection could release organisms through urine for months to years (Green *et al*., 1990). The exact period of releasing and spreading of these organisms in urine of dogs and humans is still unknown and may depend on kind of serovar (Hartman *et al*., 2010).

Water transmission is the most common way of spreading the disease (Hartman *et al*, 2010). Hot and stagnant waters are providing fair place for residence of spirochetes in environment. Spread of the disease in floods can significantly rise (Green *et al*., 1990). These organisms can be survived 180 days in humid soil and for months in surface waters (Gately, 2010). Geographical regions and seasons must be reviewed because little epidemiologic information is available. Free roaming dogs and animals with frequent contact with contaminated waters are more at risk (Greene *et al*., 1998). Some studies were showed that the probability of infection in male dogs is more (Hartman *et al*., 2010). Clinical signs in cats can be non- specific; but liver signs and renal insufficiency and hem-agglutination disorders are the most common sings (Greene *et al*., 1998, and Hartman *et al*., 2010).

Clinical signs in canine leptospirosis depend on the effects of environmental factors, host immunity, intensity and quantity (Hartman *et al*., 2010) of these organisms (Green *et al*., 1990). Canicola serovar of Leptospira interrogans, Grippotyphosa serovar of Leptospira interrogans and Icterohaemorrhagiae serovar of Leptospira kirschneri are the most common serovars that have been detected in dogs. Moreover, the two serovars; Pomona and Bratislawa are common in the U.S.A (Hartman *et al*., 2010). In addition, serovars like australis, javanica, ballum, hardjo, autumnalis and pyogenes were reported in dogs (Vijayanand *et al*., 2008). Latest serological evidences have shown that predominant serovars in dogs are changing to grippotyphosa, pomona, ratislava and autumnalis (Goldstein *et al*., 2006). Leptospirosis also was seen in cat, monkey, coyote, fox, wolf, squirrel, pig, camel, sheep, goat and cow (Rad *et al*., 2004).

The most infecting serovar in rat is icterohaemorrhagiae, in dairy cows are hardjo and pomona, in pigs are pomona and Bratislava (Gately, 2010). Leptospirosis also was seen in cats but still there is little knowledge about the rate of infection or about the causative agents (Greene *et al*., 1998). One of the most important issues about Leptospirosis is the diagnosis of the disease; because these animals can role as a potential sources for humans and other animals even after overcoming it (Hartman *et al*., 2010 and Khorami *et al*., 2010). Therefore, according to the high infection occurrence in animals and its easy transmission, especially in pet animals, the sero-epidemiologic study involving infection rate determination or sero- positivity, and also examining common serovars of each area are significant and could be helpful in diagnosing and treatment of the infection. Due to the lack of studies about the prevalence of infection and sero- prevalence in cats (Greene *et al*., 1998) and even in our region (Tabriz metropolitan), the present study can determine the sero- prevalence rates and the type of involved species of micro- organism in cats.

MATERIALS AND METHODS

In this study, serum samples of 71 cats in both household and stray groups were collected randomly during June to August of 2011. Household (pet) cats were chosen from clients of different private veterinary clinics of Tabriz and also clients of Small Animal Clinic of Azad University (Tabriz branch); stray cats were chosen from various zones of city which were caught by trapping. In each group checkups were conducted and a questionnaire for each animal concerning cats' type (household or stray) was filled. All of the household cats were commissioned to clinics for ordinary examinations, anti-parasite treatments or annual vaccination. In clinical examinations there were not any symptom representing an infectious disease and also none of the owners had any complain about the presence of disease or other suspicious signs of this illness. None of the stray cats also represented any signs related to clinical disease or infectious. For evaluation of sero- positivity, 5 ml venous blood was taken from all of the cats' available veins (especially cephalic and jugular veins). After blood sampling specimens were centrifuged 3000 RPM for 5 minutes and immediately after isolation of serum, these serums were kept in sterile micro- tubes at -20^{0C} until the end of sampling process. Samples were sent to Leptospira exploratory laboratory in veterinary college of Tehran university for completing M.A.T procedure.

Micro Agglutination Test (MAT)

In this study, in order to setting Microscopic Agglutination Test, we used 7 various serotypes of Leptospira, including Leptospira canicola, Leptospira grippotyphosa, Leptospira pomona, Leptospira icterohaemorrhagiae, Leptospira ballum, Leptospira autumnalis, Leptospira australis and Leptospira hardjo; with standard density of 2×10^{8} bacteria per ml. Before doing the test, micro- tubes were held in room temperature, in order to be thawed. In general, this test is in accordance with the suggested procedure by W.H.O (2003) and was conducted in Leptospira Exploratory Laboratory in Veterinary College of Tehran University. According to standards of W.H.O, the samples with the agglutination degrees of +1, +2 and +3 along with +4 were successively regarded as negative, suspected and positive, respectively.

In the second stage, Microscopic Agglutination test was studied in serological samples with +4 agglutination degree along with titration method in more than 1:100 of serological titers. Then dilution of 1:50 from each sample have prepared. Code of each serum samples was written on empty micro-tubes and 1000 µl of PBS solution was transferred to each micro-tube by sampler and then 20 µl of serum sample, from not diluted serum, was added; then pipetting was done several times in order to achieving an equal dilution. In this way dilution of 1:50 was achieved. For controlling the test, at the first of every test, 3 controls in 3 micro- tubes were prepared which contained negative control of serum (10µl antigen and 10µl completely negative serum which has distinguished before), Positive control (10µl antigen and 10µl completely positive serum which has distinguished before) and Antigen control (10 µl antigen and 10 µl PBS).

RESULTS

Present study was done on 71 cats in two groups of household and stray cats from June to August 2011 in Tabriz metropolis. Among 71 cats that were investigated for Leptospirosis with MAT, one of stray cats (1.4%) showed titer 1:100 against Leptospira antigens (seropositive). None of household cats showed even titer of infection to Leptospirosis (titer 1:100 or

above). The founded serum titer was against pomona serovar of Leptospira interrogans; and also none of household and stray cats showed high titer to several serovars at the same time. Among the household cats, 6 of them showed suspicious titer (+2), which 5 had titer against grippotyphosa serovar and one against to hardjo serovar. Among the stray cats 6 of them showed suspicious titer (+2), which 5 had titer against grippotyphosa serovar and 1 to hardjo serovar same as the household group. In this study 31 cats were households and 40 cats were strays. None of 31 household cats showed Positive titer (titer 1:100 or above). Statistical analysis showed that, effects of way of keeping cats as pets in house on titer of MAT is meaningless (P>0.05). It seems that this is because of the way these cats were kept. All of these cats were free roaming and therefore, make them to at risk as stray cats or because of lack of many sero- positive results. From 71 cats that were investigated in this study 34 were tomcats and 37 were female cats. Statistical analysis showed that effects of gender on titer of MAT is meaningless (P>0.05). From 31 cats of household group, unless all were kept in house they were all free roaming cats. Statistical analysis showed that effects of place of keeping on titer of MAT is meaningless (P>0.05).

DISCUSSION

Leptospirosis, which is zoonotic and mutual among vast range of animals, has worldwide spread. Because of various serovars, agent can be transferred by many domestic and wild animals, so there are various sources for the disease which has great potential to affect humans. Among the animals, canines and felines can transfer various serovars like Icterohaemorrhagiae and canicola to humans. Being zoonotic in one side and possibility of contamination in cats on the other side indicates necessity of evaluation of infection proportion of cats. Present study has done for the first time on the cats of this region to determining the kind and infection rate of Leptospirosis, in particular geographical and climatic conditions of Tabriz.

It seems that cats and dogs can be infected by 6 – 8 different serovars of Leptospiras (Hartman *et al*., 2010); but in various regions of the world, infection to different serovars has been reported, and according to reports major serovars of Leptospirosis in cats and dogs are Grripotyphosa, Canicola, Pomona, Bratislava and recently Autumnalis (Hartman *et al*., 2010 and Green *et al*., 1990). But because of the lack of infection rate reports and different kinds of serovars of the Tabriz region, this study evaluates not only the rate of infections but also the species of infectious serovars; that's because of the ability of MAT method to differentiating serovars by assaying the quantity rate of agglutination of each one; and so that we used 7 serovars which might be infectious in dogs and cats. The other reason to choose this study is the increase of infection among humans and animals, especially in humans where there are reports from some regions of our country (Iran) in last years. For example in the research done on 400 rural people living in west of Iran, they determined that 48.5% of individuals had the antibody titer against this disease, also in females it was more than males and the major serovar was hardjo (Ebrahimi *et al*., 2003). In other study done by Zakeri and *et al*. in 4 provinces of Iran, contamination of individuals to these organisms was 26.5% and the most infectious serovar was interrogans (Zakeri *et al*., 2010). Unfortunately reports showed high rates of infection in some regions like north of the country which is 1.6 of every 100 people (Esmaeili *et al*., 2009); even assaying waters of Gilan province determined that 35% of rice farms, 35% of water canals and 30% of rivers are contaminated with these organisms (Issazadeh *et al*., 2008). In a study done by Jamshidi and *et al*., in 2009 on cats, they presented the rate of contamination of cats in Tehran metropolis to Leptospirosis as 27%, in which contamination rate of household cats was higher than stray ones (Jamshidi *et al*., 2009). Such a reports show the hazardous aspects of the disease, specially the easy way of spreading of the organism among animals and humans, which can be done even by simple scratches. Therefore, evaluating the rate of contamination and prevalence of the disease in companion animals could be helpful in controlling and preserving public health.

Rates of contamination of companion animals in studies done in other countries, which are depend on climate, geographical conditions and keeping situations, have vast range; but they do not abbey a particular trend. In an investigation which was done on 230 dogs in Thailand, high percentage (83.5%) of sero- positivity was reported, in which Batavia serovar was the most prevalent (Gueguen *et al*., 2000; Scanziani *et al*., 2002). Therefore, as indicated in various studies, prevalence of Leptospirosis is depends on climate conditions, geographical region, healthcare systems and way of keeping the animals; and also as we see infectious serovars are also different. In present study evaluation of contamination of household and stray cats indicated that just one of the stray cats had titer of ≥1:100 which equal 1.4% of this cats; that report shows the least rate of contamination in Tabriz than other reports from various regions of Iran. In attentive to living situations of stray cats and high probability of their contact with other animals and contaminated stuffs, regularly their rate of contamination to Leptospirosis is high. But in this study only 1.4% of cats showed the infection and the infectious serovar was Pomona. No vaccinations had done on the cats, so existence of titer in serum, is the sign of the disease.

Various seasonal conditions, as reliable authorities have indicated, have significant effect on the rate of contamination; for example, increasing of the rate occurs after raining in spring and summer, and in attentive to that we have done our experiment in June to August, so sampling and evaluating the contamination rate in cats have set in a period with highest probability of contamination (Hartman *et al*., 2010 and Green *et al*., 1990). On the other hand, the climate like Tabriz, which is mostly cold and dry, could have negative effects on growing environment of organism, even at the time we did our sampling. Stray cats have high rate of antibody and seems that the transmission is goes from rodents (Hartman *et al*., 2010), which are carrying ballum and Icterohaemorrhagiae serovars, to cats (Jamshidi *et al*., 2009). Cats may also be in exposure to urine of dogs living around them. Loss of clinical cases may be because of cats' aversion to water and somehow their natural residency to infections (Hartman *et al*., 2010). Cats can potentially release zoonotic leptospiras by their urine for a 3 months period after being contaminated.

So in attentive to above reasons and also natural residency of cats, low serum titer of leptospirosis reported in some literatures (Greene *et al*., 1998). But it seems that it is not only reason for low infection in cats because some reports show high evidence of infection (Jamshidi *et al*., 2009). It seems that the humidity and climate are most important reasons for high percentages of infection in some areas of the worlds than cats' initially resistance to the infection.

Therefore, we can conclude that Tabriz metropolis has got minimum number of infected cats. In many articles, which are recently published, different rates of contamination of farm animals in Tabriz region has been reported (Hassanpour *et al*., 2007; Tooloie *et al*., 2008 and Hassanpour *et al*., 2009). But as such articles indicate, increase of this rate is in direct relation with crowd of animals which are kept in a place, and as the crowd of cats in a particular place regularly is not much, we can trust the result of our research; because all our household cats were kept alone and stray ones were trapped accidently from ordinary places with less population; and this is one of the merits of this study because firstly real contamination proportion of each individual cat was evaluated and secondly an accurate comparison between contamination rate of household and stray cats was done.

In the statistical analysis of MAT there were no meaningful differences between being household or stray, male or female and free roaming and restricted ($P > 0.05$). In attentive to keeping the cats in and out of the house a meaningful difference of existing of disease was not found, this was because of being free roaming of this animals, and in this situation cats will be in exposure of contaminated waters and transmitter animals like rodents, but ways of keeping have not shown any meaningful differences ($P > 0.05$); so due to results, we can conclude that less contamination of the region and natural residency of cats to the disease, makes the lack of meaningful differences logical and acceptable.
In this study we determined that contamination in Tabriz is much more less than other regions even worldwide, and this is because of less contamination rate of Tabriz in order to its climate, geographical situations and less rate of infection in other animals of this city.

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