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Short Communication

A serological survey on Leptospiral infection in companion rabbits referred to Veterinary Hospital of Shahid Chamran University of Ahvaz

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ABSTRACT

Leptospirosis is one of the major zoonotic diseases worldwide. This disease is caused by various serovars of *Leptospira interrogans sensu lato* infection. Although the rabbits are known to be one of the reservoirs and transmission sources of leptospirosis, but the status of this infection in rabbits in Iran remains unknown, so this survey was conducted to evaluate the seroprevalence of leptospiral infection in companion rabbits in Ahvaz district from September 2010 to December 2013. Blood samples were taken from 68 companion rabbits, referred to veterinary hospital of shahid Chamran University of Ahvaz. Sera were screened for antibodies against serovars of *Canicola*, *Icterohaemorrhagiae*, *Grippotyphosa*, *Ballum*, *Hardjo, Pomona, Australis and Tarassovi* using the microscopic agglutination test (MAT). From a total of 68 rabbits, 19 (27.94%) were serologically positive for the serovars of *L. Icterohaemorrhagiae*, *Tarassovi, Grippotyphosa*, *Hardjo, Pomona and Australis*. The positive titers were detected between 1:100 to 1:200 dilutions. The predominant titers were directed against serovars of *Icterohaemorrhagiae* (36.84%; 7 out of 19) and *Tarassovi* (36.84%; 7 out of 19) and followed by *Hardjo* (21.05%; 4 out of 19), *Australis* (10.53%; 2 out of 19), *Pomona* (5.26%; 1 out of 19) and *grippotyphosa* (5.26%; 1 out of 19). Antibodies against more than one serovar (mixed infections) were detected in three samples (as *Pomona+Hardjo* and *Icterohaemorrhagiae+ Tarassovi*). The prevalence was significantly higher in adult rabbits above 3 years (37.04%; 10 out of 27) and between 1-3 years (36.36%; 8 out of 22) compared with rabbits less than 1 years (5.26%; 1 out of 19) ($P<0.05$). Prevalence was higher in males rabbits (30.77%; 12 out of 39) than females (24.14%; 7 out of 29), and in the season of summer (31.25%; 5 out of 16), but the difference was not significant relative to host gender and season ($P>0.05$). This survey indicated that serovars of *L. Icterohaemorrhagiae and Tarassovi* were predominant in the rabbits of this area and can be a source of infection for humans. The obtained results provide useful information on the epidemiology of leptospirosis in this species, which has not been previously investigated.

Keywords: Leptospirosis; Seroprevalence; Rabbit; Zoonosis; Ahvaz; Iran

INTRODUCTION

Leptospirosis is an important zoonotic disease that is distributed worldwide. *Leptospira* is a gram-negative, helical shaped bacterium from the group spirochetes. There are more than 230 distinct leptospiral serovars recognized and these are arranged in 23 serogroups. In mammals, leptospirosis is transmitted either by direct contact with infected animals, during their daily activities or by exposure to water or soil contaminated

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with the urine of infected animals (Hartmann and Greene 2005). Chronically infected animals may remain carriers for years to life and serve as reservoirs of the infection for other animals and humans (Greene *et al* 2006). Role of rabbits as carriers of *Leptospira* has been investigated in some countries and it has been reported to carry different pathogenic leptospiral serovars (Hartmann and Greene 2005), however the role of them as a source of human infections has not been investigated in Iran. Leptospirosis has become an important public health problem in Asia (Patil *et al* 2014). Previous studies have been shown that the prevalence of antibodies against *leptospira* in rabbits population and other species are quite variable, depending on the method of research, the number of animals and geographic area (Greene *et al* 2006, Goncalves *et al* 2010). Rabbits may be exposed to the infected urine of cohabiting rabbits that may contain different serovars. Most animals remain carriers long after the initial infection and continue to excrete bacteria into the water sources and soil. Long-term survival of pathogenic leptospires outside the host requires a warm and moist environment with near-neutral pH. In the United States and Canada, a positive correlation has been reported between the prevalence of leptospirosis and the average rainfall (Hartmann and Greene 2005). Leptospirosis is often diagnosed by serological tests because culturing is expensive and has many disadvantages such as taking between 3 to 12 weeks to isolate the leptospires (Emmett *et al* 1971, Niwetpathomwat & Doungchawee 2006). Serological testing is the laboratory procedure most frequently used to confirm the clinical diagnosis, to determine prevalence, and to conduct epidemiological studies. Leptospiral antibodies appear within a few days of onset of illness and persist for weeks or months and in some cases, years. A variety of serological tests have been developed which show varying serogroup and serovar specificities (Hartmann & Greene 2005, Greene *et al* 2006). Two noticeable tests in veterinary diagnosis are the microscopic agglutination test (MAT) and enzyme-linked immunosorbent assay (ELISA). MAT is sensitive and specific, and is considered to be the standard serological test for the diagnosis of leptospirosis. It is widely used to detect the anti-*Leptospira* antibodies in serum samples (National Veterinary Services Laboratories 1987). Most studies conducted on leptospirosis in Iran and other countries, have used MAT to identify the prevailing *Leptospira* serovars among humans and animals (Avizeh *et al* 2008, Patil *et al* 2014). The endpoint is defined as that dilution of serum that shows 50% agglutination, leaving 50% free cells compared with a control culture diluted 1/2 in phosphate buffered saline. The result of the test may be reported as the endpoint dilution of serum (e.g. 1/100 or 1/400) or as a titer that is the reciprocal of the endpoint serum dilution (e.g. 100 or 400). Many laboratories perform a screening test at a final serum dilution of 1/100 and then retest sera with titers of ≥100 to determine an endpoint using doubling dilutions of sera beginning at 1/100 through to 1/12,800 or higher (Tilley & Smith 2004). Antibodies against *Leptospira* have been detected in serum samples of humans and other animals of Ahvaz district (Haji Hajikolaei *et al* 2005, Mosallanezhad *et al* 2011, Mosallanezhad *et al* 2013). However, the status of leptospirosis in rabbits of Ahvaz district has remained unknown, so the aim of this survey was to provide preliminary information on the seroprevalence of leptospiral infection among rabbits found in this area. Such information among the populations of animals and identifying the predominant carrier species are important in control and prevention programs. To our knowledge, this research is the first report of leptospiral infection in companion rabbit's population from Iran.

MATERIALS AND METHODS

Blood samples were collected from 68 companion rabbits, referred to veterinary hospital of Shahid Chamran University of Ahvaz, from September 2010 to December 2013. The studied rabbits were classified according to age, sex and season. They were divided into three age groups, two sex-based and four season-based groups. Age determination was accomplished

based on dental formulary and obtained information's from the owners. At the time of blood collection, all the animals seemed to be healthy and no clinical signs of leptospirosis were observed. Two milliliters of blood was collected from the marginal or jugular vein of each rabbit. Serum samples were kept at -20ºC until serological tests were done. In history, there was not vaccine administration against *leptospira*. They were not examined on necropsy for gross evidence of the disease. Using the MAT, sera were tested for antibodies against eight live antigens of *L. interrogans* (serovars of *Canicola*, *Icterohaemorrhagiae*, *Grippotyphosa*, *Ballum*, *Hardjo, Pomona, Australis and Tarassovi*). 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 saline (PBS) in a microtiter plate (Greiner) up to 1:800 dilutions, starting from an initial 1:50 dilution. Then, 10 µL of the serum dilution was added to 10 µL of the appropriate antigen on a microscopic slide. This was placed in a plate containing a moist paper to avoid evaporation and incubated at 30°C for 90 minutes. 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. Results were considered positive when agglutination was found at least 50% or more of leptospires.

Statistical Analysis. To determine whether there were any statistically significant relationships between the prevalence of positive cases and other factors such as age, sex and different season, data were examined using Chi-square analysis and Fisher's exact test with a confidence interval of 95%. Differences were considered significant when $P< 0.05$.

RESULTS AND DISCUSSION

From a total of 68 rabbits, 19 (27.94%) were serologically positive for the serovars of *L. Icterohaemorrhagiae*, *Grippotyphosa*, *Hardjo, Pomona, Australis and Tarassovi*. The positive titers were detected between 1:100 to 1:200 dilutions. The predominant titers were directed to serovars of *Icterohaemorrhagiae* (36.84%; 7 out of 19) and *Tarassovi* (36.84%; 7 out of 19) followed by *Hardjo* (21.05%; 4 out of 19), *Australis* (10.53%; 2 out of 19), *Pomona* (5.26%; 1 out of 19) and *grippotyphosa* (5.26%; 1 out of 19). Antibodies against more than one serovar (mixed infections) were detected in three samples (as *Pomona+Hardjo* in one case and *Icterohaemorrhagiae+ Tarassovi* in two cases). As expected, prevalence was significantly higher in adult rabbits above 3 years (37.04%; 10 out of 27) and between 1-3 years (36.36%; 8 out of 22) compared with rabbits less than 1 years (5.26%; 1 out of 19) ($P<0.05$). Prevalence was higher in males rabbits (30.77%; 12 out of 39) than females (24.14%; 7 out of 29), and in the season of summer (31.25%; 5 out of 16), but the difference was not significant relative to host gender and season ($P>0.05$). In other seasons, (winter, spring and autumn) seroprevalence were 25%, 29.41%, and 26.67% respectively. These results are summarized in Tables 1 and 2.

The overall seroprevalence of leptospiral infection was 27.94% among 68 rabbits. In the present study, sampling was conducted for nearly three years in the studied rabbits, so the results can be considered as representative for the rabbit's population in the Ahvaz district, Iran. It seems that the rabbit population of the Ahvaz district may have been exposed to one of these reservoirs (especially farm animals and other rodents) or to environmental contamination of the urine of different species, located in recreational areas. This is the first published serological study of *leptospirosis* in companion rabbit's population in Iran, in order to determine the predominant serovars of *leptospira* using MAT. MAT is considered to be a sensitive and specific serological test for diagnosis of *leptospirosis* and is called as standard serologic means; nevertheless it is less useful in the diagnosis of chronic disease in maintenance hosts (Greene *et al* 2006). Vaccination

history must also be considered in the interpretation of MAT results, as widespread vaccination contributes significantly to the number of seropositive animals and may mask the presence of chronic infections, particularly with serovar of *L. hardjo*. Iran is known to be one of the countries in Asia, possessing endemic areas for leptospirosis (Patil *et al* 2014). Little information is available about the status of leptospirosis in our country in terms of its prevalence and incidence among rodents, particular rabbits. In America, rabbits are small mammals that are more common than dogs and cats as pets. Until 2006, about 6.2 million domestic rabbits have been existed at 1.6 percent of America's family (Sampasa-Kanyinga *et al* 2012), but there is not exact information about the number of companion rabbits in Iran.

Table 1. Prevalence of *L. interrogans* with different serovars among rabbits (n=68) of different ages and sexes, referred to veterinary hospital of Shahid Chamran University of Ahvaz, from September 2010 to December 2013.

Age / Sex	< 1 years		1–3 years		> 3 years	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Male	10	1	9	5	8	6
Female	8	0	5	3	9	4
Total = 68	18	1	14	8	17	10

Table 2: Prevalence of *L. interrogans* with different serovars among rabbits (n=68) of different ages and seasons, referred to veterinary hospital of Shahid Chamran University of Ahvaz, from September 2010 to December 2013.

Age / Region	< 1 years		1–3 years		> 3 years	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Spring	5	-	3	2	4	3
Summer	4	1	4	2	3	2
Autumn	5	-	2	1	4	3
Winter	4	-	5	3	6	2
Total	18	1	14	8	17	10

In our study, *Leptospira interrogans* serovars of *Icterohaemorrhagiae* (36.84%) and *Tarassovi* (36.84%) which had the highest reactivity were considered to be the most important infecting serovars. Rabbits are usually kept in groups and crowding places and this increases the likelihood of infection in the early years. Evidence strongly suggests that rabbits are one of the most important reservoirs of leptospires nevertheless; the results of the present study do not indicate the sources of infections in the rabbits. Although these animals may harbor the organisms, they do not get sick or die of leptospirosis. However, they may become chronically infected and continuously shed the organisms for more than seven months or years (Greene *et al* 2006). As previously mentioned, among 68 serum samples, nineteen had antibodies against at least one serovar of *Leptospira*. A possible reason for this finding might be the fact that rabbits used in this study had been infected with these serovars before keeping in the house. Finding antibodies with different serovars in the rabbits such as *L. Icterohaemorrhagiae*, *Tarassovi, Grippotyphosa*, *Hardjo, Pomona and Australis*, suggest that rabbits might have been previously exposed in contact with other infected animal species in this area. Studies have shown that isolated populations of mammals are important in the maintenance of unusual serovars. The epidemiology of leptospirosis is complex and varies significantly in different environmental settings (Natarajaseenivasan *et al* 2002). Obtained results confirm that the seroprevalence of leptospiral infection among rabbit's population is different not only between countries but also between various areas of a country. These differences can be explained by epidemiological diversity of the *leptospira* infection in different countries. Significant variation is seen in the duration of different serovars survivals according to the pH of soil and water (Hartmann and Greene 2005). In a survey on fifty rabbits, *L. interrogans* antibodies were demonstrated in 77% of the serum collected, of which 21% had significant titers. Serotypes most frequently encountered were *ballum, australis, icterohaemorrhagiae, canicola* and *grippotyphosa* (Emmett *et al* 1971). Isolation of grippotyphosa was made from 8% of the kidneys collected. In our study,

among the six serovars that were identified, serovars of *Icterohaemorrhagiae* and *Tarassovi* were the most predominant in Ahvaz district, nevertheless traditionally, serovars of *canicola* and *icterohaemorrhagiae* are considered to be the most significant serovars in animal's worldwide (Greene *et al*., 1998). In the present survey, the prevalence of leptospiral infection was 30.77% and 24.14% in males and females, respectively. There was no significant difference in positive titer prevalence between distinctive sexes. The obtained results also indicated that there was no significant relationship between different seasons. The prevalence of leptospiral infection in dogs, cats and rats (*Rattus rattus*) were reported to be 5.4% (8/149), 4.9% (5/102) and 3.33% (4/120) respectively in the Ahvaz district (Avizeh *et al* 2008, Mosallanejad *et al* 2011, Mosallanejad *et al* 2013), which is less than our data (27.94%) (13, 14). High prevalence of the leptospiral infections has been reported in other animals in Ahvaz, such as buffalo (58.73%), cattle (53.79%) horse (27.88%) and donkey (40.00%) (Haji Hajikolaei *et al* 2005). Higher prevalence in buffalo and cattle is probably due to their additional access to the contaminated environments or waters. Our data is nearly similar to infection rate in horse and donkey. Crowding of animals (particular rabbits) can enhance the spread of infection. In addition, rabbits are adapted to live in different areas and pathogen transmission appears to be more in these habitats. For these reasons, rabbits have a higher chance of being exposed to leptospires, especially in the early years of life which can infect them through direct contact with the mucosal membranes (Greene *et al* 2006). The climatic conditions (warm and humid) in our area appear to be suitable for the survival of the *leptospira*. Temperature of Ahvaz can be up to 50°C in summer. Hot weather and west soil can increase the survival of leptospires, which may explain the higher prevalence of infection compared with other areas (Haji Hajikolaei *et al* 2005). Because of the importance of water as a means of spreading infection, new cases are most likely to occur in wet seasons and low lying areas, especially when contamination and susceptibility are high. In serological tests for leptospirosis, the results often indicate infections by more than oneserovar, which may be due to mixed serovar infections. In the present study, antibodies against more than one serovar were detected in three samples (as *Pomona+Hardjo* in one case and *Icterohaemorrhagiae+ Tarassovi* in two cases). The prevalence of infection and titers of 1:100 to 1:200 revealed that leptospiral infection was relatively high in the rabbits of Ahvaz district. Although serological surveys may provide an approximation for the exposure level, they do not provide information regarding the number animals actively shedding leptospires. In some countries, leptospirosis is endemic and infection is much more common than clinical disease. This is particularly so in Egypt where there the literature indicates widespread serological prevalence without a significant rate of clinical disease (Felt *et al* 2011). In the present study, we believe that companion rabbits may be the source of different serovars of *leptospira*. In the previous studies, carriage of leptospirosis was found to be correlated with the age (Greene *et al* 2006). In our survey, prevalence was significantly higher in adult rabbits above 3 years (37.04%) and between 1-3 years (36.36%) compared with rabbits less than 1 year (5.26%). The demonstration of antibodies in adult rabbits more than juveniles indicates that exposure level to infection is more in higher ages. The results of our study provide useful information on the epidemiology of leptospirosis in Iran, which until now was not well studied; in addition, studies with larger sample sizes on leptospirosis among rabbits in other areas of Iran would be beneficial in determining the transmission cycle of leptospirosis and the status of this zoonosis. The observations provided may also be useful in formulating leptospirosis prevention and control measures and guidelines in Iran and other countries with similar conditions. The temperature condition required for maximal leptospiral survival may explain the differences in the leptospiral

prevalence in various parts of the world (Hartmann and Greene 2005). The incidence of disease seems that has been decreased in areas where vaccination is carried out for protection of rabbits against *leptospirosis*. The recognized primary reservoir hosts for infecting serovars of rabbits include the rat (*icterohaemorrhagiae*), dog (*canicola*), vole (*grippotyphosa*), cow (*hardjo*) and pigs (*pomona*) (Hartmann and Greene 2005, Greene *et al* 2006). The presence of antibodies in rabbits is a public health concern due to the close contact between them and humans, which provides a link between an environmental reservoir and humans. We hope that in near future, this and other similar projects provide the basis of an epidemiologic surveillance program in companion rabbits of Ahvaz district, adapted to the particular conditions of our country, which will establish the basis for prevention and control of these kinds of emerging diseases.

Ethics

I hereby declare all ethical standards have been respected in preparation of the article.

Conflict of Interest

Hereby, I declare "no conflict of interest exists" regarding submitted article.

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