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A serological survey on Leptospiral infection in squirrels and hamsters in Ahvaz district, South-West of Iran

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Keywords

Leptospirosis; seroprevalence; squirrel; hamster; Ahvaz

Abstract

Leptospirosis is a zoonotic infectious disease of worldwide distribution. The infection is caused by various serovars of *Leptospira* interrogans sensu lato. Although the squirrels and hamsters are considered to be the reservoir or maintenance host of *Leptospira*, but little is known about the status of leptospirosis in these animals. This survey was conducted to evaluate the seroprevalence of Leptospiral infection in squirrels and hamsters in Ahvaz district, South-West of Iran. Blood samples were taken from 35 squirrels and 35 hamsters. Sera were screened for antibodies against serovars of *L. canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. ballum*, *L. hardjo*, *L. pomona*, *L. australis* and *L. tarassovi* using the microscopic agglutination test (MAT). From a total of 35 squirrels, three cases (8.57%) were serologically positive for the serovars of *L. grippotyphosa* (2.86%), *L. pomona* (2.86%) and complex of *L. hardjo* + *L. canicola* + *L. grippotyphosa* + *L. pomona* (2.86%). Positive results were detectable at serum dilutions of 1:100 to 1:400. Seroprevalence did not show a significant difference for age and sex in the studied squirrel ($p > 0.05$). From a total of 35 hamsters, six cases (17.14%) were serologically positive. The predominant titers were directed against serovars of *L. grippotyphosa* (5.71%), *L. grippotyphosa* + *L. pomona* (2.86%), *L. pomona* (2.86%), *L. icterohaemorrhagiae* (2.86%) and *L. canicola* (2.86%). The positive results had 1:100 serum dilutions. Prevalence was significantly higher in adult hamsters above one year compared to hamsters less than one year ($p < 0.05$). The seroprevalence was more in male hamsters (23.53%) than females (11.11%), but the statistical analysis did not show a significant difference ($p > 0.05$). This survey indicated that serovars of *L. grippotyphosa* and *L. pomona* were predominant. The results provide useful information on the seroprevalence of leptospirosis in squirrels and hamsters of Ahvaz district.

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Abbreviations

L.: *Leptospira*
MAT: Microscopic Agglutination Test
ELISA: Enzyme-Linked Immunosorbent Assay

Introduction

Leptospirosis, the most widespread zoonosis in the world, is an emerging public health problem, particularly in large urban centers of developing countries. The infection is caused by various serovars of *Leptospira* interrogans sensu lato. The *Leptospira* genus comprises approximately 20 species and more than 300 serovars (Greene et al., 2012; Haake and Levett, 2015). Among wildlife species, rodents are the most important reservoirs for *Leptospira* spp. and may transfer infection to livestock, companion animals and humans. Squirrels and hamsters have been adopted as popular small house pets. As rodent species may be carriers of distinct Leptospiral serovars in different geographic areas, knowledge of the prevalent serovars and the reservoirs are essential to understand the epidemiology of the disease. The transmission of leptospirosis between human and animals is through the infection of the renal tubule and excretion of infectious agents in the urine of carrier animals. Urine shed from carriers can result transmission of the infection via the mucous membranes or indirectly via contaminated water eg: drinking or swimming in canals or rivers (Tilley and Smith, 2000; Haake, 2006; Matsui et al., 2015). Long-term survival of pathogenic agents outside the host requires a warm and moist environment with near-neutral pH. The disease is seasonal, with peak incidence in summer or fall. Clinical signs associated with leptospirosis can range from insignificant to death (Marinho et al., 2009). Yet little epidemiological research has been conducted on rodents in the world. It was shown that 45% of fox squirrels were positive for Leptospiral infection in Colorado, USA (Dirsmith et al., 2013). Leptospirosis was diagnosed in the patients exposed to southern flying squirrels imported from the United States to Japan (Masuzawa et al., 2006). Longitudinal studies have been conducted that the main risk factors of leptospirosis are contact to rodent as well as rice farming and keeping animals in Khuzestan Province. Another frequent risk factor in this area is swimming in rivers or brooks (Alavi et al., 2014). Diagnosis of leptospirosis is often made by serological tests because culture is expensive and has many disadvantages as it takes between 3 to 12 weeks. A wide variety of serological tests, which show varying degrees of serogroups and serovar specificity, have been described (Greene et al., 2012). Two rapid tests have an important 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. It is widely used as the reference test for leptospirosis diagnosis and is considered to be a useful tool in epidemiologic studies or surveillance on leptospirosis. The endpoint is defined as that dilution of serum that shows 50% agglutination, leaving 50% free cells compared to control culture diluted 1/2 in phosphate buffered saline. 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 to 1/12,800 or higher (Hajikolaei et al., 2007; Greene et al., 2012). Most studies conducted on leptospirosis have used the MAT to identify the prevailing *Leptospira* serovars among humans and animals in Iran and other countries. Antibodies against *Leptospira* have been detected in serum samples of humans and other animals in Ahvaz district (Hajikolaei et al., 2007; Mosallanejad et al., 2013). Prevalence of the infection is not clear in the squirrels and hamsters in Iran, so the aim of this survey was to provide preliminary information on the seroprevalence of Leptospiral infection among these animal species found in Ahvaz district, Iran. Knowledge of the predominant *Leptospira* serovars in animal carriers and reservoirs may be of epidemiological value in monitoring their circulation, determining potential exposure to animals, implementing prevention and intervention measures. To our knowledge, this research is the first report of Leptospiral infection in the population of squirrels and hamsters in Iran.

Materials and Methods

This study was conducted in Ahvaz district, South-West of Iran, which is located at the height of 12 meters above sea with warm and humid climate. In this study, seventy animals (35 squirrels and 35 hamsters) were examined. Twenty-one squirrels and twenty-seven hamsters were referred to the Veterinary Hospital of Shahid Chamran University of Ahvaz. Some squirrels (fourteen) and hamsters (eight) were also taken from pet shops. The animals were divided into different groups, based on age and gender. Age of animals were provided by the owners and calculated by dental formula (Hudson and Romagnano, 2010). At least two milliliter of blood samples were collected from the femoral veins of each animal (between November 2014 to August 2015). All animals appeared healthy and showed no clinical signs of a disease. None of them had been vaccinated against *Leptospira*. They were anaesthetized by administration of ketamine (10 mg/kg) and acepromazine (0.15 mg/kg). The serum samples were stored at -20°C until analysis. Using the MAT, sera were tested for antibodies against eight live antigens of L. interrogans (serovars of *L. canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. ballum*, *L. hardjo*, *L. pomona*, *L. australis* and *L. tarassovi*). The tests were performed in the Research Laboratory (Faculty of Veterinary Medicine, University of Tehran, Iran) mainly as described by Turner "MAT meth-

Table 1
Prevalence of Leptospiral infection among the squirrels (n=35) based on age and gender in Ahvaz District, South-West of Iran, by MAT.

	Age (≤ 1 year)		Age (> 1 year)	
	Negative	Positive	Negative	Positive
Male	8 (53.33%)**	1 (6.67%)**	6 (40%)**	0
Female	8 (40%)**	0	10 (50%)**	2 (10%)**
Total (35)	16 (94.12%)*	1 (5.88%)*	16 (88.89%)*	2 (11.11%)*

*(95% CI for proportion: 0-17.9% for squirrels) * percent for age, ** Percent for Gender.

od" with some modifications. All serum samples were two-fold serially diluted in phosphate buffer solution (PBS) in a microtiter plate 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 antigen on a microscopic slide. Finally, the slides were 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 pathogenic agents (Greene, 2012).

Statistical analysis

To examine whether there were any statistically significant relationships between the prevalence of positive cases and other factors such as age and gender. The 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

From a total of 35 squirrels, three cases (8.57%) (0-17.9%; 95% CI for proportion) were serologically positive for the serovars of *L. grippotyphosa* (2.86%), *L. pomona* (2.86%) and complex of *L. hardjo* + *L. canicola* + *L. grippotyphosa* and *L. pomona* (2.86%). An antibody against more than one serovar was detectable only in one sample. The positive results were detectable at serum dilutions of 1:100 to 1:400. The seroprevalence of Leptospiral infection was 5.71% and 2.86% in female and male squirrels, respectively. Among the affected squirrels, two out of eighteen animals had age above one year and one out of seventeen had age below one year (Table 1). The seroprevalence did not show a significant difference for age and gender ($p > 0.05$). In a total of 35 hamsters, six cases (17.14%) (4.6-29.6%; 95% CI for proportion) were serologically positive. The predominant titers were directed against serovars of *L. grippotyphosa* (5.71%), *L. grippotyphosa* + *L. pomona* (2.86%), *L. pomona* (2.86%), *L. icterohaemorrhagiae* (2.86%) and *L. canicola* (2.86%). An antibody against more than one serovar was detectable only in one sample. The positive results had 1:100 serum dilutions. The prevalence was significantly higher in adult hamsters above one year compared with hamsters less than one year ($p < 0.05$). The seroprevalence was more in male hamsters than females, but the statistical analysis did not show a significant difference between them ($p > 0.05$). The results are summarized in Tables 1 and 2.

Discussion

Overall, the present survey showed that 8.57% of the squirrels and 17.14% of the hamsters were positive for Leptospiral infection in Ahvaz district, South-West of Iran. Iran is known to be one of the countries in Asia, possessing endemic areas for leptospirosis (Talebkhan Garoussi et al., 2006). Nevertheless, there is not enough information about Leptospirosis in Iran regarding the prevalence and incidence of Leptospirosis in rodents, circulating *Leptospira* species, reservoirs, transmission, and Leptospiral pathogenicity. In recent years, interest in the maintenance of exotic animals has grown in our country, so knowledge of zoonotic diseases is important. The purpose of this study was

Table 2
Prevalence of Leptospiral infection among the hamsters (n=35) based on age and gender in Ahvaz District, South-West of Iran, by MAT.

	Age (≤ 1 year)		Age (> 1 year)	
	Negative	Positive	Negative	Positive
Male	11 (64.71%)**	0	2 (11.76%)**	4 (23.53%)**
Female	9 (50%)**	0	7 (38.89%)**	2 (11.11%)**
Total (35)	20 (100%)*	0	20 (100%)*	6 (40%)*

*(95% CI for proportion: 4.6-29.6% for hamsters) * percent for age, ** Percent for Gender

to obtain information on leptospirosis in terms of its prevalence among the squirrels and hamsters in Ahvaz district. In the present research, sampling was conducted for nearly ten months, so the results can be considered representative for the population of squirrels and hamsters in this area.

Evidence strongly suggests that rodents are one of the most important reservoirs of leptospirosis. L. interrogans and L. borgpetersenii species are widely distributed among rodents, and strain typing has confirmed rodents as reservoir for human leptospirosis. Although these animals may harbor the organisms, they do not get sick or die of leptospirosis. However, rodents may become chronically infected and continuously shed the organisms for more than seven months, thereby contaminating the environment and making it possible for the pathogenic agents to come in contact with other animals or human (Hudson and Romagnano, 2010; Cosson et al., 2014).

The MAT is the most common serological test which is used for the diagnosis of leptospirosis (Hajikolaei et al., 2007). In serological tests for leptospirosis, the results often indicate infection by more than one serovar, which may be due to mixed serovar infections. As mentioned previously, of these seventy serum samples, two had antibody against more than one serovar. A possible reason for this finding may be that the animals used in this study have been previously infected with these serovars (such as *L. grippotyphosa* and *L. pomona*). They have probably a strong tendency to persist in the renal tubules of squirrels and hamsters. Finding of antibodies for other serovars that do not usually exist in these species, such as *L. canicola*, *L. hardjio* and *L. icterohaemorrhagiae*, suggests that they may have been in close contact with other animal species such as canine, equine, bovine and even wildlife in this area, needs further investigation. Longitudinal studies have shown that isolated populations of mammals are important in the maintenance of unusual serovars, such as the carriage of serovar *L. bim* by Mus musculus in Barbados (Matthias and Levett, 2002). The results of several articles confirm that the prevalence of Leptospiral infection in rodents is different not only between countries but also between different areas within a country (Greene, 2012). These results can be explained by diversity in the epidemiology of the Leptospiral infection in different countries. Significant variation is seen in the duration of survival of different serovars according to the pH of soil and water. In the United States and Canada, a positive correlation has been reported between the prevalence of leptospirosis and average rainfall (Tilley and Smith, 2000). The prevalence of infection was reported between 17.2-31.8% in hamsters of Czech Republic. The serovars of *L. grippotyphosa* and *L. pomona* were dominant in the examined hamsters (Greene, 2012). Similarly, our results showed that *Leptospira* interrogans (serovars of *L. grippotyphosa* and *L. pomona*) had the highest reactivity compared to other serovars in hamsters. They are considered to be the most important infecting serovars.

Leptospiral infection was found between 6-13% in rodents of Germany (Mayer-Scholl et al., 2014). The prevalence of infection has been reported 30.2% in rodents of China and up to 40% in Italy (Vitale et al., 2007; Wang and He, 2013). It was stated that the prevalence of positive leptospirosis tests in rodents was 15.9, 2.6, and 2.6% among *Rattus norvegicus*, *R. rattus*, and *Apodemus sylvaticus*, respectively (Esfandiari et al., 2015). In another survey on rats and mice in Mashhad, the infection rate was significantly higher in rats than house mice (Talebkhan Garoussi et al., 2006). In the present study, the prevalence of Leptospiral infection was more in female squirrels and male hamsters; nevertheless, there was no significant difference between various genders.

As mentioned earlier in this report, previous seroepidemiologic studies in Iran have detected antibodies against several *Leptospira* serovars in human and animal serum samples (Hajikolaei et al., 2005; Mosallanejad et al., 2013; Mosallanejad et al., 2015). The prevalence of Leptospiral infection were reported to be 5.4% (8/149) and 4.9% (5/102) in dogs and cats in Ahvaz district, respectively, which is less than half to our data (12.86%) (Avizeh et al., 2008; Mosallanejad et al., 2011). These results suggest that the animals such as squirrels and hamsters have increased access to contaminated environments. In addition, these animals are maintained densely in bird stores in Iran and this condition increases risk of infection. For these reasons, the squirrels and hamsters have a higher chance of being exposed to pathogenic agents that can infect them 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. The higher prevalence of Leptospiral infection in other animals in Ahvaz district, such as cattle (53.79%), horse (27.88%), buffalo (58.73%) and donkey (40.00%), is probably due to their greater access to stagnant water and contaminated environments. These animals live as a group near water, which can increase the likelihood of infection. Crowding of animals can also enhance spreading of infection (Hajikolaei et al., 2005). Although serological surveys may provide estimation of the exposure level of these animals, it does not provide information regarding how many of them are actively shedding agents and posing a potential zoonotic risk in this area. In the present study, although our sample size was relatively small, on the basis of the typing of isolates, we believe that the squirrels and hamsters can be the source of different serovars of pathogens. The climatic conditions in this area (warm and humid) appear to be suitable for the survival of the *Leptospira*.

In the previous studies, carriage of leptospirosis was found to be correlated with the age. In their research, the serological positivity had increased proportionately to the weight, i.e. to the age of the animals (Greene, 2012). In the present survey, the prevalence was significantly higher in adult hamsters above one year compared to age less than one year. The demonstration of antibodies in adult hamsters more than young's indicates that exposure level to

infection is more in higher ages. The results of our study provide useful information on the Leptospiral infection in the squirrels and hamsters in Iran, which until now are not well studied, as well as, studies with larger sample sizes on leptospirosis among squirrels, hamsters, humans, and other animals in other areas of Iran will be beneficial in determination of the transmission cycle of leptospirosis and the status of this zoonosis. The observations provided in our survey may also be guidelines for other countries with similar conditions. The positive results were detectable at serum dilutions of 1:100 to 1:400 for different serovars. The prevalence of infection (12.86%) reveals that Leptospiral infection is relatively average in the squirrels and hamsters in Ahvaz district. The presence of antibodies in these species can be a public health concern due to the close contact between them and human, which provides a link between an environmental reservoir and humans (Levett, 2004). We hope that, in the near future, this and other similar projects will provide the basis of an epidemiologic surveillance program in the squirrels and hamsters in Ahvaz district, South-West of Iran, adapted to the particular conditions of our country, which will establish the basis for prevention and control of these kinds of infectious diseases.

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