

# A Serological Survey on Leptospiral Infection Among Wild Rats (*Rattus rattus*) of Ahvaz District, Southwest of Iran: A Preliminary Study

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**Background:** Leptospirosis is an important zoonotic disease caused by pathogenic *Leptospira* which can infect both humans and animals. This disease is caused by various serovars of *Leptospira interrogans sensu lato* infection. Rats are known to be one of the most important reservoirs and transmission sources of leptospirosis. However, the status of leptospirosis in wild rats has been unknown in many areas of Iran.

**Objectives:** This study was conducted to evaluate the seroprevalence of leptospiral infection in wild rats (*Rattus rattus*) in Ahvaz district (southwest of Iran) from October 2009 to November 2011.

**Materials and Methods:** Rats were trapped alive, anaesthetized, and blood-extracted by cardiac puncture. Serum samples were collected from 120 wild rats and screened for different leptospiral serovars using the microscopic agglutination test (MAT). The rats were classified according to sex, season and region of capture. The results were analyzed by Chi-square analysis and Fisher's exact test.

**Results:** From a total of 120 rats, 4 (3.33%) were serologically positive for the *L. gryppotyphosa* serovar. All positive titers were detected at 1:100 dilutions. Antibodies against more than one serovar were not detected in any sample. The prevalence of leptospiral infection was 2.5% and 0.83% in male and female rats, respectively. There was no significant difference in positive titer prevalence between different sexes, seasons and areas ( $P > 0.05$ ).

**Conclusions:** This survey indicated that serovars of *L. grippotyphosa* are prevalent in the rats population of this area and can be a source of infection for humans. The results of the present study provide useful information on the epidemiology of leptospirosis in this species, which was not well studied before.

**Keywords:** Leptospirosis; Seroprevalence; Wild Rat (*Rattus rattus*); Zoonosis; Ahvaz; Iran

## 1. Background

Leptospirosis is an important zoonotic disease with worldwide distribution, caused by the pathogenic spirochetes of the *Leptospira* genus. Role of rats has been investigated as carriers of *Leptospira* in some countries and different species of rats have been reported to carry different pathogenic leptospiral serovars (1). Maintenance hosts usually develop chronic infection of the renal tubules at an early age and remain asymptomatic while excreting leptospores for long periods of time (2). Three species of the *Rattus* genus have been reported from Iran: the brown rat (*Rattus norvegicus*), the black rat (*R. rattus*), and the Himalayan rat (*R. pectoris*) (3). Population of Wild rats is a potentially important reservoir host for the transmission of zoonotic parasites such as *Leptospira*. Leptospirosis has become an important public health problem in Asia.

Previous studies have shown that the prevalence of an-

tibodies against *Leptospira* in the wild rats population is quite variable, depending on the method of research, number of animals studied and geographic area (4). The role of wild rats as a source of human infections has been rarely investigated in Iran. In a survey in Iran (Mashhad), the seroprevalence was 21.73% (5). Humans and animals can be exposed to leptospirosis during their daily activities or at work through environments contaminated with leptospores-containing rat urine in urban and rural areas (4). Rats may be exposed to infected urine of co-habiting rats 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 (2). Long-term survival of pathogenic leptospores outside the host requires a warm and moist environment with near-neutral pH. Leptospirosis is often diagnosed by serological tests because culturing is expensive and has many disadvantages such as taking between 3 to 12 weeks

### Implication for health policy/practice/research/medical education:

Leptospirosis is a zoonotic disease of global importance caused by pathogenic *Leptospira* which can infect both humans and animals. Rodents, especially rats, are considered to be the most important reservoirs or maintenance hosts of *Leptospira*.

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to isolate the leptospires (2, 4).

A variety of serological tests have been developed which show varying serogroup and serovar specificities (1, 2). 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 (1). Most studies conducted on leptospirosis in Iran and other countries, have used MAT to identify the prevailing *Leptospira* serovars among humans and animals. Antibodies against *Leptospira* have been detected in serum samples of humans and other animals of Ahvaz district (6). However, the status of leptospirosis in wild rats of Ahvaz district has remained unknown in Iran.

## 2. Objectives

The aim of this survey was to provide preliminary information on the seroprevalence of leptospiral infection among wild rats found in Ahvaz, southwestern Iran. Such information among the populations of animals and identifying the predominant carrier species are important in control and prevention programs.

## 3. Materials and Methods

Wild rats (*R. rattus*) were trapped alive, anaesthetized by Halotan, and blood-extracted by cardiac puncture during October 2009 to November 2011. Two milliliters of blood was collected from the heart of each rat. Serum samples were collected from 120 wild rats (*R. rattus*) in different areas of Ahvaz district, Southwestern Iran. The samples were kept at -20°C until consumption. The rats were divided into two sex-based, four season-based and five area-based groups. At the time of blood collection, all animals seemed to be healthy and no clinical sign of leptospirosis was observed. Using the MAT, sera were tested for antibodies against seven live antigens of *L. interrogans* (serovars pomona, canicola, hardjo, balum, icterohaemorrhagiae, gryppotyphosa and australis).

The tests were performed in the Leptospiral Research Laboratory (Faculty of Veterinary Medicine, Tehran University of Medical Sciences, Tehran, Iran) mainly as described by Turner "MAT method" with some modifications

(National Veterinary Services Laboratories) (7). All serum samples were two-fold serially diluted in phosphate buffer saline (PBS) in a microtiter plate (Greiner, Bio-One, Frickenhausen, Germany) 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, Japan). 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 leptospires (6, 7).

### 3.1. Statistical Analysis

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

## 4. Results

Four out of the 120 rats (3.33%) were serologically positive for *L. interrogans* serovar *gryppotyphosa*. All positive titers were detected at 1:100 dilutions. Antibodies against more than one serovar were not detected in any samples. The prevalence of leptospiral infection was 2.5% and 0.83% in male and female rats, respectively. In the present study, all the infected rats seemed to be adult. They were not examined on necropsy for gross evidence of the disease. The prevalence was higher for the rats captured in the summer (3.85%) as well as the ones captured from then East region (7.69%), but distribution of leptospiral infection was not significantly different in the positive titer prevalence between different sexes, seasons and capture areas ( $P > 0.05$ ). In other seasons, winter, spring and autumn, prevalence was 3.45%, 2.86%, and 3.33%, and in other regions, north, west, south, and center, it was 0%, 0%, 4.54%, and 3.85%, respectively. These results are summarized in Table 1.

**Table 1.** Prevalence of *L. interrogans* serovar *gryppotyphosa* Infection Among Wild Rats (n = 120) Captured in Different Seasons and Regions of the Ahvaz District, Iran, 2009 - 2011

Area	Spring, No.		Summer, No.		Autumn, No.		Winter, No.	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
North	7	0	5	0	7	0	7	0
East	8	1	4	0	6	0	6	1
West	6	0	5	0	4	0	5	0
South	6	0	6	1	5	0	4	0
Center	7	0	5	0	7	1	6	0
Total	34	1	25	1	29	1	28	1

## 5. Discussion

The results showed that 3.33% of the wild rats were seropositive for the *L. gryppotyphosa* serovar in the Ahvaz district. Iran is known to be one of the countries in Asia, possessing endemic areas for leptospirosis (5). However, little information is available about the status of leptospirosis in our country in terms of its prevalence and incidence among rodents, particular wild rats. Sampling was conducted in wild rats (*R. rattus*) for nearly two years, so the results can be considered as representative for the wild rat population in the Ahvaz district, Iran. Evidence strongly suggests that rats are one of the most important reservoirs of leptospires (2). 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 (1).

Our survey is the second published study on leptospirosis in Iran considering the leptospirosis diagnosis using MAT. MAT is the most common serological test for the diagnosis of leptospirosis; nevertheless it is less useful in the diagnosis of chronic disease in maintenance hosts (7). In our study, *L. interrogans* (serovar of *gryppotyphosa*) which had the highest reactivity was considered to be the most important infecting serovar. As previously mentioned, among 120 serum samples, four had antibodies against only one *Leptospira* serovar. A possible reason for this finding might be the fact that rats used in this study had been previously infected with this serovar; however, these findings suggest that this serovar probably has a strong tendency of persistence in the renal tubules of wild rats in this area.

Finding antibodies in the rats of other serovars such as Canicola, Bratislava, Hardjio and Pomona, which are not usually found in this species, suggests that rodents might have been in close contact with other animal species such as canine, equine, and even bovine in this area. Studies have shown that isolated populations of mammals are important in the maintenance of unusual serovars, such as the carriage of serovar bimby house mice (*Mus musculus*) in Barbados (8). The epidemiology of leptospirosis is complex and varies significantly in different environmental settings. Obtained results confirm that the prevalence of leptospiral infection in rats is different not only between countries but also between various areas of a country. Significant variation is seen in the duration of different serovars survivals according to the pH of soil and water (2). In the United States and Canada, a positive correlation has been reported between the prevalence of leptospirosis and the average rainfall (4).

The prevalence of leptospiral infection among rat populations in different areas of the world has been reported as: 21.73% in Mashhad (Iran) (5), 25% and 20% in Colombia (9), 17.3% in Kelantan, 18.4% in Terengganu (Malaysia) (10) and 26% in Egypt (11). These differences can be explained by epidemiological diversity of the *Leptospira* infection in

different countries. Kawabata et al. reported the first record of *L. borgpetersenii* in the Amami Islands, Japan (12). In a survey in Mashhad, the infection rate was significantly higher in the rats compared to the house mice. However, there were no significant differences among various infection titers (5). In the present study, the prevalence of leptospiral infection was 2.5% and 0.83% in male and female rats, respectively. There was no significant difference in positive titer prevalence between distinctive sexes, seasons and areas ( $P > 0.05$ ). The prevalence of leptospiral infection in dogs and cats were reported to be 5.4% (8/149) and 4.9% (5/102) respectively in the Ahvaz district, which is nearly similar to our data (3.33%) (13, 14).

These results suggest that animals such as rats have limited access to contaminated environments. In addition, rats are adapted to live under the buildings and pathogen transmission appears to be slower in these habitats. For these reasons, rats have a lower chance of being exposed to leptospires, which can infect the animal through direct contact with the mucosal membranes. Nevertheless, the results of the present study do not indicate the sources of infections in the rats. Higher prevalence of the leptospiral infections in other animals in Ahvaz, such as cattle (53.79%), horse (27.88%), buffalo (58.73%) and donkey (40.00%) (6), is probably due to their additional access to the stagnant water and contaminated environments. These animals live in groups near water, which can increase the likelihood of infection.

Crowding of animals can also enhance the spread of infection. Although serological surveys may provide an approximation for the exposure level of these animals, they do not provide information regarding the number rats actively shedding leptospires in this area. In the present study, we believe that wild rats may be the source of serogroup of *Grippotyphosa*. The climatic conditions in this area (warm and humid) appear to be suitable for the survival of the *Leptospira*. We observed that the wild rats obtained in Ahvaz were species of *R. rattus*. In the previous studies, carriage of leptospirosis was found to be correlated with the species of rats and its age (5). In our survey, all infected rats seemed adults, however, we were not able to determine the age of the captured rats as well. Therefore, it would be useful if the rats ages could be identified in the future investigations.

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 rats in other areas of Iran would be beneficial in determining the transmission cycle of leptospirosis and the status of this zoonosis. The observations provided in our survey 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 the mentioned different parts of Iran.

Temperature of Ahvaz can be up to 50°C in summer and hot weather and dry soil and decrease the survival of leptospire (13), which may explain the lower prevalence of cases compared to Mashhad (5). In serological tests for leptospirosis, the results often indicate infections by more than one serovar, which may be due to mixed serovar infections, but in the present study, antibodies for more than one serovar were not found in any of the serum samples. The prevalence of infection and titers of 1:100 revealed that leptospiral infection was relatively low in the wild rats of Ahvaz district. The obtained results also indicated that there was no significant relationship between different sexes, seasons and areas of captured infected rats.

The presence of antibodies in rats is a public health concern due to the close contact between rats and humans, which provides a link between an environmental reservoir and humans. Wild rats and other rodents are the main reservoirs for this serovar (*L. gryppotyphosa*) and this suggests that the rat population of Ahvaz may have been exposed to one of these reservoir species directly or through environmental contamination by the urine of these animals. Rats should be eliminated by the local animal control centers, particularly in the areas in which contact with other domestic mammalian species is probable (2, 15). We hope that in near future, this and other similar projects provide the basis of an epidemiologic surveillance program in wild rats of Ahvaz, Southwestern Iran, adapted to the particular conditions of our country, which will establish the basis for prevention and control of these kinds of emerging diseases.

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### Authors' Contribution

None declared.

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There is no financial interest to disclose.

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