

Association of Prevalent *Leptospira* Species with Different Rodents of Three Northern Provinces in Iran Using Microscopic Agglutination Test

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Abstract

Purpose: Leptospirosis as a zoonotic disease is caused by *Leptospira* bacteria. Transmission occurs by contact with contaminated biological fluids of the infected animals. Rodents are major sources of infection for humans or other animals. The disease is distributed mainly in tropical regions with rainfall like northern part of Iran. The aim of this study was to find association of prevalent *Leptospira* species with different rodents of three Northern Provinces in Iran using microscopic agglutination test (MAT).

Methods: In this study, 404 rodents were captured alive at 10 different parts of each Mazandaran, Gilan and Golestan Provinces. Identification of the infecting serovars and the antibody titers were done by MAT in the sera samples using a panel of 20 strains of live *Leptospira* spp. as the source of antigens.

RESULTS: Antibodies against one or more serovars were detected in 94 (23.27%) sera at dilution $\geq 1:200$ and 76.73% were detected to be negative. The prevalent *Leptospira* serovars were detected as *L. autumnalis* (25.53%), *L. serjoehardjo* (24.47%) and *L. cynopteri* (6.38%). The majority of rodents were identified during this study in three provinces included *Rattus norvegicus* (67.33%), *Apodemus sylvaticus* (13.86%) and *Rattus rattus* (13.61%). The common prevalent rodent in three provinces was *Rattus norvegicus*, which was associated with *L. serjoehardjo* in Mazandaran (81.8%), *L. autumnalis* in Gilan (67.2%) and *L. canicula* in Golestan (50.0%).

Conclusion: The dominant serovars of leptospira were *L. autumnalis*, *L. serjoehardjo* and *L. cynopteri* and the most prevalent rodents as reservoir were *Rattus norvegicus*, *Apodemus sylvaticus* and *Rattus rattus* in three Northern provinces of Iran. The results indicated a moderate prevalence of leptospirosis in rodents during this study in north of Iran. This study provided the first epidemiological data about the association between leptospirosis with rodents in Iran.

Keywords: Mazandaran. Gilan. Golestan. Iran. *Leptospira*. Leptospirosis. MAT. Rodent

Introduction

Leptospirosis is one of the most important common zoonotic diseases. It has a wide distribution in the world specially temperate and humid climate ranges. *Leptospira* species can be transferred directly by skin or indirectly from livestock to human. Clinical symptoms of this disease are including icteric and anicteric forms. Infected animals stay almost as a carrier in their lifetime and they excrete bacteria in their urine, periodically. The most of the pathogen spp. can survive in the environment for a long time and they can cause infection in humans and animals through damaged skin [1, 2].

In Iran, temperate and humid climate ranges from the plains along Caspian Sea border to northern foothills of Alborz Mountain. In this region, rice planting is the predominant occupation. Most peasants are farmers and keep one or more livestock in their houses. In mentioned region, surface waters or rivers are mainly used for agriculture. So this condition is appropriate for spreading of leptospirosis to susceptible hosts [3].

Following description of leptospirosis by Weil (1886) [3], sero-bacteriological has been the method of choice for detection in most countries [4]. Some studies have indicated high prevalence of the disease in different countries among domestic and wild mammals [1]. The first case of leptospirosis was reported in 1956 in Iran. Several other reports were published later on throughout Iran [5, 6]. In these studies, more than thousand sera samples from cattle, sheep and camels herd were analyzed using microscopic agglutination test (MAT) and the results demonstrated that cows (31%) and sheep (17%) were infected with *L. grippityphosa*, *L. pomona* or *L. icterohaemorrhagiae* [7]. Ever since 1997 where the disease was reported for first time from peasants in Rasht city of Gilan province (North of Iran), number of cases have been reported especially during the rice cultivation season every year. Due to increase in the dissemination of information about the disease among the local physicians and laboratories, a rise in the suspected patients leptospirosis in June and July of 1998 was reported in comparison with the previous years [3].

Diagnosis of leptospirosis is based on the serological findings or cultured samples of blood, cerebrospinal fluid and urine. Isolation of *Leptospira* spp. does not occur in most diagnostic laboratories because of difficulties of this method such as bacterial frail nature, difficulty of the isolation media, the cost, and the prolonged incubation period. Therefore, serological assay plays an axial role in the detection of the disease. Recently, the most method is based on the detection of specific serum antibodies. World health organization (WHO) has defined MAT as a more reliable diagnostic test as compare to the other tests present and also as the gold standard method for identification of *Leptospira* [8]. High sensitivity of the MAT and its specificity for serovars are advantages of this method [9].

In northern part of Iran (Mazandaran, Gilan and Golestan provinces), for reduction of leptospirosis, proper identification of infected animals is necessary using serological tests. The purpose of this study was to identify the association between prevalent *Leptospira* species with different rodents of three Northern Provinces in Iran using MAT.

Materials and Methods

Study area and sample collection

This descriptive cross-sectional study was performed during the summer of 2013 in three provinces northern part of Iran, Mazandaran, Gilan and Golestan, according to the area map (**Figure 1**). In this study, active colonies of rodents in

10 major parts of each of the three provinces and a total of 404 rodents were trapped alive. They were determined and categorized by the key characteristics such as gender, genus, species, different locations and topological situation.

Preparation of blood samples

Animals were terminally anaesthetized and 5 mL blood taken into a syringe by cardiac puncture. Blood samples were put into tubes without heparin for separating serum for MAT. The non-heparin samples were centrifuged at 3000 g for 10 min at room temperature. The sera samples were kept in sterile 1.5 mL micro-tubes at -20°C until used. Death occurs after bleeding of rodents.

Microscopic Agglutination Test (MAT)

MAT was performed on the sera samples collected using 20 live leptospiral strains as antigens. The strains belong to the serogroups including *Australis* (strain Jez Bratislava), *Autumnalis* (Akiyami A), *Ballum* (Mus 127), *Bataviae* (Swart), *Canicola* (Hond Utrecht IV), *Icterohaemorrhagiae* (RGA), *Grippotyphosa* (Moskova V), *Hebdomadis* (Hebdomadis), *Javanica* (Poi), *Pomona* (Pomona), *Pyrogenes* (Pyrogenes) and *Semarang* (Patoc I). MAT was applied at doubling dilutions starting from 1 in 20. Positive samples were titrated to their end point. All the strains were maintained in EMJH medium with periodical subculture. Seven days old cultures free from contaminations were utilized for the performance of MAT [11, 12]. The samples were considered positive if at least 50% of agglutination of *Leptospira* in a dilution test serum of $\geq 1:200$ were observed. Sera with positive results were titrated against reacting antigens in serial two-fold dilutions from 1:200 to 1:1600.

Data Analysis

Statistical analysis was performed in two stages using SPSS version 17. The first was a descriptive which aimed to characterize the study sample. The second stage was aimed to correlate all statistical variables and parameters.

Results

Antibodies against one or more serovars were detected in 94 (23.27%) sera at dilution $\geq 1:200$ and 310 (76.73%) were detected to be negative. The distributions of serotypes of *Leptospira* by MAT in the ten vicinity parts of three cities of the Mazandaran Province (Sari, Nour and Noshahr), ten centers of Gilan Province (Roodsar, Langerood, Lahijan, Rasht, Fooman, Some'esara, Masouleh, Bandar Anzali, Talysh and Astara) and ten centers of the Golestan Province (Gorgan, Kordkuy, Kohneh, Azadshahr, Minoo Dasht, Kalaleh, Gonbad Kavoods, Maraveh Tappeh, Incheh Boroun and Bandar Torkaman) were 21.19%, 21.19% and 29.41%, respectively. The most common *Leptospira* serotype was detected to be *L. serjoehardjo* in Mazandaran Province ($p > 0.05$), *L. autumnalis* (7.9%) in Gilan Province and *L. canicola* and *L. serjoehardjo* (7.8%) in Golestan Province. In these provinces, a total of 15 species of *Leptospira* were found and *L. autumnalis*

had highest distribution in them. The overall majority of *Leptospira* serovars were belong to *L. autumnalis* (25.53%), *L. serjoehardjo* (24.47%) and *L. cynopteri* (6.38%) (**Table 1**).

The overall prevalent rodents were identified during this study in three provinces were *Rattus norvegicus* (67.33%), *Apodemus sylvaticus* (13.86%) and *Rattus rattus* (13.61%). The common prevalent rodent in three provinces was *Rattus norvegicus*, which was associated with *L. serjoehardjo* in Mazandaran (81.8%), *L. autumnalis* in Gilan (67.2%) and *L. canicola* in Golestan (50.0%) (**Table 2**).

Discussion

The marginal area of Caspian Sea, northern Iran includes three provinces, Mazandaran, Gilan and Golestan has plain, temperate climates and humid conditions which is appropriate for *Leptospira* infection. Rodents are important reservoir hosts for pathogenic serovars of *Leptospira* and the most common source for human leptospirosis [12]. The finding of the present study showed that *L. autumnalis*, *L. serjoehardjo*, *L. cynopteri* and *L. canicola* are the most common serovars amongst rodents in three provinces of northern Iran, while, the most serotype that had been reported in other studies was *L. canicola*, suggesting that this serovar is more prudent to be maintained in the rodent populations [13]. The high prevalence of *L. canicola* serovar in this study is in accordance with the results of other serological study conducted in recent years in other parts of Iran [14]. In a study on 2002 has been reported that *L. canicola* and 3 syndromes (Weil's, atypical pneumonia and aseptic meningitis) have a significant relationship [15]. Moreover, the results of the present study indicated that rodents may have an important role in transmission of the leptospirosis in the rural regions in these provinces. The possibility that rodents may be a maintenance host for *L. autumnalis* suggests that cross-infection between rodents and domestic animals may occur in the rural regions.

The results of this study revealed a prevalence of 29.4% *Leptospira* infection among rodents in Golestan province. In the majority of studies, it has been shown that farmers are usually involved in disease in comparison with other professions. In the study of Perret et al. (2005) in Chile, the incidence rate of the disease among the farmers was reported 72% [16]. Slack et al. (2010) had mentioned that the most important risk factors of this disease were occupational factors, direct or indirect contact with the animals or their dead bodies, with grass and bushes, swimming, hunting, aquatic sports, and traveling to hot and rainy areas, suggesting the important influence of the type of occupation on the disease outbreak [1, 17].

MAT is considered the gold standard reference test for serological diagnosis of leptospirosis which is more sensitive than PCR and nested PCR, also, culture is more specific than the others [18]. The first comprehensive study about leptospirosis in Iran was reported by Rafyi & Maghami in 1957 on sera samples of cattle, sheep and camels using MAT in the Razi Vaccine and Serum Research

Institute in Iran. Their results indicated 31% of cows and 17% of sheep were infected with *L. grippityphosa*, *L. omona* or *L. icterohaemorrhagiae* [19]. In addition to MAT, several culture and molecular methods have been tested for detection of leptospira including dot blot hybridization method with marked probes for P32 and biotinylated [20, 21].

Although, the MAT is still the gold standard test for serological diagnosis of the leptospirosis, the interpretation of MAT results could be complicated because of cross-reactivity amongst different serogroups, particularly during the acute stage of the disease in the clinical samples [22]. Overall, the present study support the idea that leptospirosis is still one of the important factors of economic losses in the plain region. The trend of seroprevalence of leptospirosis in northern Iran is increasing and more investigations are needed to be conducted in this regard and the other part of Iran in order to clarify the epidemiological picture of leptospirosis in the country.

Conclusion

The dominant serovars of leptospira were *L. autumnalis*, *L. serjoehardjo* and *L. cynopteri* and the most prevalent rodents as reservoir were *Rattus norvegicus*, *Apodemus sylvaticus* and *Rattus rattus* in three Northern provinces of Iran. The results indicated a moderate prevalence of leptospirosis in rodents during this study in north of Iran. This study provided the first epidemiological data about the association between leptospirosis with rodents in Iran.

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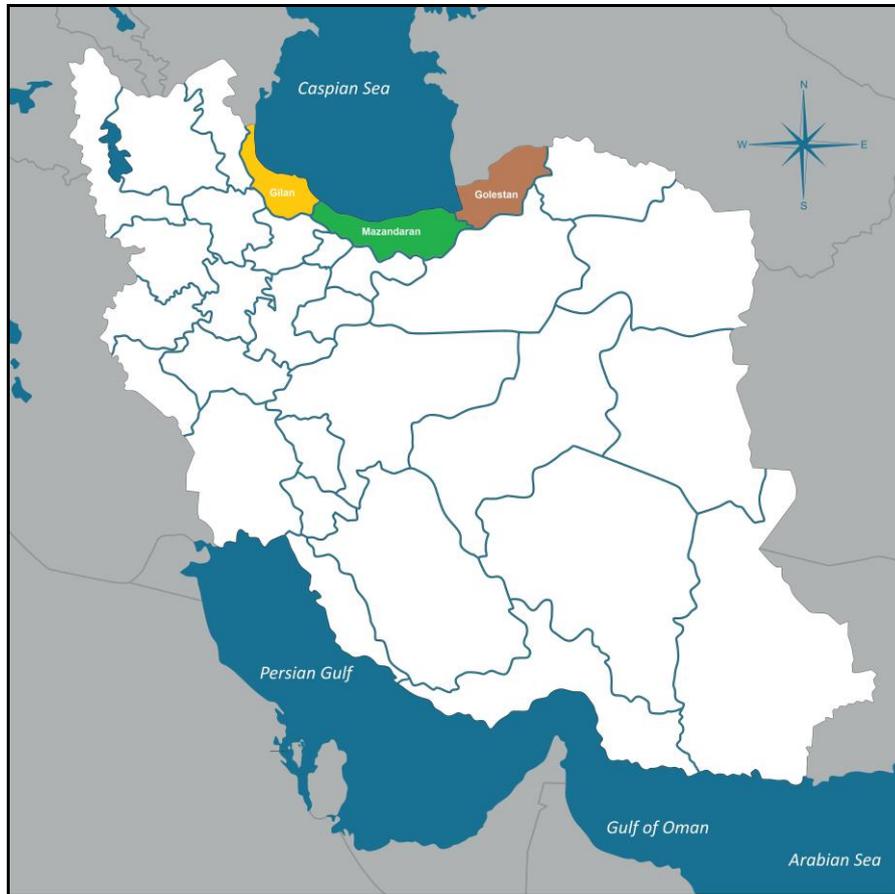


Figure 1. Iran map and study areas including Gilan, Mazandaran and Golestan Provinces

Province	Frequency of <i>Leptospira</i> serotypes [No. (%)]															Total
	1	2	3	4	5	7	8	9	10	11	12	13	14	15	16	
Mazandaran	5 (20.8)	1 (11.1)	2 (100)	11 (47.8)	5 (71.4)	1 (25)	3 (42.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	3 (50)	32/151 (21.19)
Gilan	12 (50)	0 (0)	0 (0)	4 (17.4)	2 (28.6)	2 (50)	2 (28.6)	2 (100)	1 (100)	3 (60)	0 (0)	1 (100)	0 (0)	0 (0)	3 (50)	32/151 (21.19)
Golestan	7 (29.2)	8 (88.9)	0 (0)	8 (34.8)	0 (0)	1 (25)	2 (28.6)	0 (0)	0 (0)	2 (40)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	30/102 (29.41)
Total	24	9	2	23	7	4	7	2	1	5	1	1	1	1	6	94/404 (23.27)

Table 1. Frequency of different serotypes of *Leptospira* in the three northern provinces of Iran
1; *L. autumnalis*, 2; *L. canicola*, 3; *L. grippotyphosa*, 4; *L. serjoehardjo*, 5; *L. icterohaemorrhagiae copenhagen*, 6; *L. icterohaemorrhagia icterohaemorrhagiae*, 7; *L. baiium*, 8; *L. australis*, 9; *L. pyrogenes*, 10; *L. sejiroesejroe*, 11; *L. javanica*, 12; *L. bataviae*, 13; *L. sejiroewolffi*, 14; *L. tarrasovie*, 15; *L. lai*, 16; *L. cynopteri*

Province	Rodent types	Frequency of <i>Leptospira</i> serotypes [No. (%)]															Total	
		Negative	1	2	3	4	5	7	8	9	10	11	12	13	14	15		16
Mazandaran	<i>Rattus norvegicus</i>	92 (77.3)	5 (100)	0 (0)	1 (50)	9 (81.8)	2 (40)	1 (100)	3 (100)							0 (0)	3 (100)	116 (76.8)
	<i>Rattus rattus</i>	5 (4.2)	0 (0)	0 (0)	0 (0)	1 (9.1)	2 (40)	0 (0)	0 (0)							1 (100)	0 (0)	9 (6)
	<i>Apodemus sylvaticus</i>	22 (18.5)	0 (0)	1 (100)	1 (50)	1 (9.1)	1 (20)	0 (0)	0 (0)							0 (0)	0 (0)	26 (17.2)
	Total	119	5	1	2	11	5	1	3							1	3	151
Gilan	<i>Rattus norvegicus</i>	80 (67.2)	8 (66.7)			3 (75)	2 (100)	1 (50)	1 (50)	2 (100)	0 (0)	2 (66.7)		1 (100)			2 (66.7)	102 (67.5)
	<i>Rattus rattus</i>	25 (21)	2 (16.7)			0 (0)	0 (0)	1 (50)	1 (50)	0 (0)	1 (100)	1 (33.3)		0 (0)			1 (33.3)	32 (21.2)
	<i>Apodemus sylvaticus</i>	9 (7.6)	2 (16.7)			1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)			0 (0)	12 (7.9)
	<i>Arvicola</i>	4 (3.4)	0 (0)			0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)			0 (0)	4 (2.6)
	<i>Mus musculus</i>	1 (.8)	0 (0)			0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)			0 (0)	1 (.7)
	Total	119	12			4	2	2	2	2	1	3		1			3	151
Golestan	<i>Rattus norvegicus</i>	37 (51.4)	3 (42.9)	4 (50)		5 (62.5)		1 (100)	1 (50)			2 (100)	1 (100)		0 (0)			54 (52.9)
	<i>Rattus rattus</i>	10 (13.9)	0 (0)	2 (25)		2 (25)		0 (0)	0 (0)			0 (0)	0 (0)		0 (0)			14 (13.7)
	<i>Apodemus sylvaticus</i>	12 (16.7)	2 (28.6)	2 (25)		1 (12.5)		0 (0)	0 (0)			0 (0)	0 (0)		1 (100)			18 (17.6)
	<i>Nesokia indica</i>	8 (11.1)	1 (14.3)	0 (0)		0 (0)		0 (0)	1 (50)			0 (0)	0 (0)		0 (0)			10 (9.8)
	<i>Cricetulus migratus</i>	3 (4.2)	0 (0)	0 (0)		0 (0)		0 (0)	0 (0)			0 (0)	0 (0)	0 (0)	0 (0)			3 (2.9)
	<i>Rhombomys opimus</i>	2 (2.8)	1 (14.3)	0 (0)		0 (0)		0 (0)	0 (0)			0 (0)	0 (0)		0 (0)			3 (2.9)
	Total	72	7	8		8		1	2			2	1		1			102

Table 2. Frequency of different rodents associated with serotypes of *Leptospira* in the three northern provinces of Iran 1; *L. autumnalis*, 2; *L. canicola*, 3; *L. grippotyphosa*, 4; *L. serjoehardjo*, 5; *L. icterohaemorrhagiae copenhagen*, 6; *L. icterohaemorrhagiae icterohaemorrhagiae*, 7; *L. baiium*, 8; *L. australis*, 9; *L. pyrogenes*, 10; *L. sejroesejroe*, 11; *L. javanica*, 12; *L. bataviae*, 13; *L. sejroewolffi*, 14; *L. tarrasovie*, 15; *L. lai*, 16; *L. cynopteri*