

## Distribution of Human Leptospirosis in Guilan Province, Northern Iran

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### Abstract

**Background:** Leptospirosis is the most widespread zoonosis in the world and is more prevalent in tropical and temperate regions. Guilan Province in north of Iran, is an endemic region of human leptospirosis. Since diagnosis of leptospirosis according to clinical symptoms is very difficult due to lack of characteristic pathogenomic sign(s), laboratory support is necessary. **Methods:** In 2003, we obtained blood samples from patients hospitalized in main general hospitals of Guilan Province and were suspected as having leptospirosis according to their clinical presentations. We examined 995 sera by a commercial IgM and IgG ELISA kit to find positive cases. **Results:** 62.7% of positive cases were male and about 86% of them were farmer. High distribution rate of leptospirosis was seen in middle-aged people (65% in 20-50 years old). **Conclusion:** It seems that leptospirosis has a high occurrence in major cities and is mostly distributed in warm months of the year. Demographic analysis of the results indicates that leptospirosis is typically a rural and an occupational disease in the area.

**Keywords:** *Leptospirosis, Zoonosis, ELISA, Iran*

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### Introduction

Leptospirosis is a bacterial disease caused by pathogenic *Leptospira*s. It can infect people through contact with an environment contaminated by the urine of a shedder host, such as rodents. This disease is one of the important zoonoses in the world. It is more prevalent in tropical and temperate regions especially in areas with moist climate and is usually endemic in these regions. Rodents as well as wild and domestic animals are main reservoirs and human is accidental host (1-3).

Most of animals play a carrier role for a long time after infection and excrete bacteria in their urine, periodically. *Leptospira*s can live in

water and wet soil and may penetrate into the body of another host (animal or human) through skin cut. Leptospirosis can be confirmed by laboratory testing of a blood or urine sample. However, clinical diagnosis is difficult during the early stages of the disease, when it may be confused with many other common febrile illnesses, such as dengue fever, malaria, typhoid, and viral hepatitis. Diagnosis of leptospirosis is often made by serological tests, because culture is both slow and expensive. Performance of the reference serological test, the Microscopic Agglutination Test (MAT), requires significant expertise, and rarely performs by routine diagnostic laboratories (4-9). Several alternative serologi-

cal methods for the early diagnosis of leptospirosis have been described and ELISA is a common method for detection of immunoglobulin M (IgM) antibodies (8-14). In some studies, sensitivity of ELISA is reported from 77.8% to 100% (14-17).

In recent years, several rapid ELISA kits for the easy and reliable detection of anti-*Leptospira* antibodies in the blood of patients have become commercially available. We performed this study by applying a commercial ELISA assay to test sera of patients suspected of leptospirosis to find positive cases.

### Materials and Methods

In collaboration with Guilan Province Health Center, 10 ml vein blood was taken from 995 patients with a history and clinical manifestations suggestive of leptospirosis, based on physician diagnosis, and according to the symptoms asserted by WHO guidance for diagnosis (11), admitted to general hospitals of Guilan Province, northern Iran. All sera were stored in -20 °C before examination. IgM and IgG titers were determined using two separate kits and according to manufacturer procedure (Serion ELISA, Classic *Leptospira* IgM and IgG kits Institute Virion/ Serion GmbH, Wurzburg, Germany). We obtained average OD of two positive samples of any kit, multiplied it in two numbers related to high and low standard cut off which were mentioned in manual of any kit,

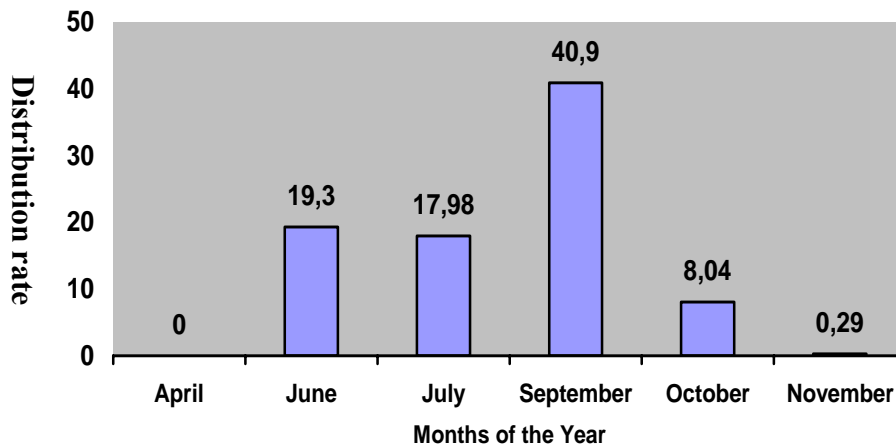
to obtain high and low cut off scores. All specimens, whose OD scores were higher than high OD cut off, were regarded positive and vice versa. Specimens which were positive in IgM-ELISA assay despite any result in IgG- ELISA assay were considered positive cases indicating recent infection and all specimens which were positive in IgG- ELISA assay but negative or borderline in IgM-ELISA assay were regarded as an indicate of previous infection. Those sera, which were negative in IgM assay and negative or borderline in IgG assay, were considered as negative cases.

### Results

Results of IgM and IgG-ELISA assays of blood samples are depicted in Table 1. Eighty one sera were at borderline in IgM-ELISA assay, which were excluded in our study. 63.7% of positive cases were male. Distribution of positive cases in terms of different months of the year is depicted in Fig.1

**Table 1:** Results of IgM and IgG ELISA assays of blood samples

IgM/ IgG	n	%
IgM and IgG positive	353	35.5
IgM positive IgG negative	178	17.9
IgM negative IgG positive	22	2.2
IgM negative / IgG negative	216	21.7



**Fig. 1:** Distribution of leptospirosis cases according to different months of the year

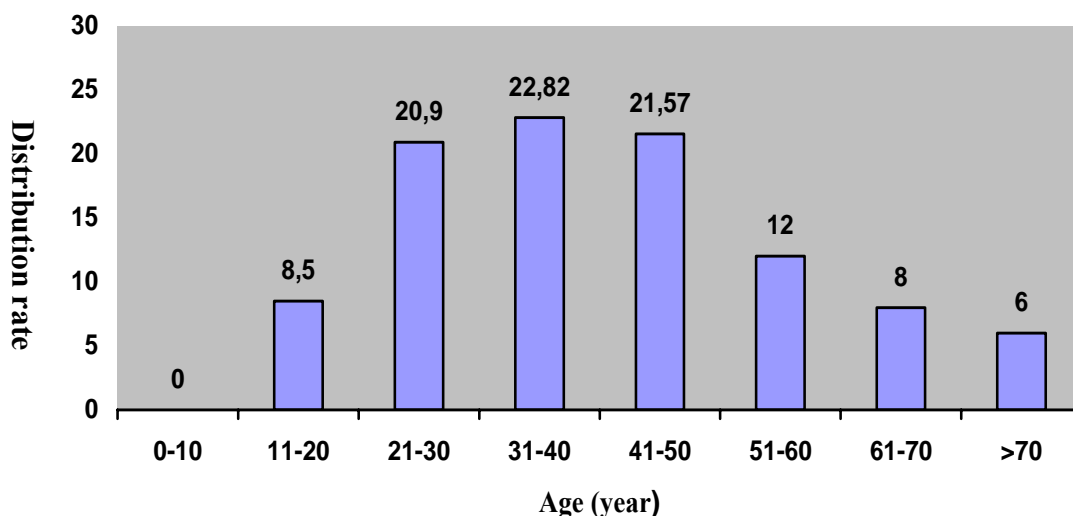


Fig. 2: Distribution of leptospirosis cases in terms of age.

Job distribution of positive cases is depicted in Table 2. Farmers were the most exposure group.

Table 2: Distribution of leptospirosis cases in terms of job

Jobs	Farmer	Worker	Employee	Student	Driver	Cooker	Fisherman	Unemployed
Distribution rate (%)	86.4	5.3	3.8	2.4	1.0	0.5	1.0	2.4

Finally geographical distribution of positive cases is illustrated in Table 3. It indicates that the disease is more widespread in flat area of Guilan, especially in the most crowded towns having numerous villages.

Table 3: Geographical distribution of leptospirosis cases in Guilan Province

City	n	%
Rasht	233	36.0
Somesarah	218	34.7
Lahijan	57	9.1
Masal	20	3.1
Anzali	19	2.9
Shaft	19	2.9
Astaneh	18	2.8
Foman	12	1.8
Langarud	10	1.5
Rudsar	10	1.5
Rezvanshahr	9.0	1.4
Talesh	8.0	1.2
Siahkal	7.0	1.0
Rudbar	2.0	0.3
Amlash	1.0	0.1
Rostamabad	1.0	0.1

## Discussion

In some parts of Iran, climatologic, environmental, and socioeconomic conditions are highly favorable for incidence of human leptospirosis. In Guilan Province, there are two different areas: highland and flat. In rural regions of flat area, rice farming is the main job of most people and cattle husbandry is the second activity. They usually keep cattle to take dairy products, horse to carry heavy bodies, and dog for security. All domestic animals are released in farms between two agricultural seasons. There are also plenty of wild animals, specially jackals, boars, and rodents who live near villages. Most of them may be carriers of *Leptospira*. Men and women work together in rice farming and cattle husbandry. Rice paddies should be wet most of the time and are usually irrigated by small channels originated from rivers and ponds. Indeed, in this area, many patients with clinical signs and symptoms compatible with leptospirosis attend hospitals every year.

By analyzing demographical features of positive cases, we found that human leptospirosis was widespread in rural regions of flat area of Guilan Province especially in big and more populated towns with most incidents in farmers (86.40%). More incidence rate in warm months of year is compatible with rice farming period and more occurrence in middle aged people who are very active in rice farming and animal keeping, and finally more incidence rate in men (1.7 time more than women) are other proofs that the disease is typically a rural disease with high relation to rice farming. Men and women work in rice farms in different style. Women are high active in rice farming and animal keeping but they work more protected, traditionally, so they are in low risk of exposure.

Our findings were similar to some other studies (18, 19). Chumakov studied leptospirosis incidence in Russia between 1962 until 2004 and found more occurrences of the disease in warm months of year (2-4 times) and more occurrences in 20-50 years old people (20). Strock and colleagues studied the incidence of leptospirosis in Guadelope and found that the disease was more common in rural regions and in rainy season (21). In Thailand more incidence of leptospirosis in farm workers was found (22). We found a high distribution rate in September (41%) which was time of rice harvesting, traditionally a male activity who used to work less protected with bare feet to walk easier and bare hands to work better. Feet and hands can be injured by leaves of adult rice that has very sharp edges. Using horses to carry harvested rice bunches and swimming in ponds especially in warm days is very common, so risk of exposure is very high in men.

Distribution rate in three big towns of the area that include too many villages and are more crowded was very high (totally 88.5%). So low distribution rate (totally 11.5%) in other regions which include a wide area with 9 town and many villages is not logical and we guess three factors may be involved:

Less familiarity of physicians with leptospirosis;

Less collaboration of health workers in detecting and reporting this disease;

Less exposure of people for unknown reasons that must be detected by more studies, especially animal sources and irrigation ways.

As a conclusion, our results demonstrated that leptospirosis had a high incidence in main cities of Guilan Province and was mostly transmitted in the warm months of year. Statistical analysis of the results indicates that leptospirosis is typically a rural and an occupational disease in the area.

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