

## Serological Examination of Groups under Leptospirosis Risk: The Artvin / Turkey Example

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**Summary:** Leptospirosis is a zoonosis of the world prevalent caused by infection with *Leptospira interrogans*, a pathogenic spirochete and it is an important health problem in Turkey. This study was designed to determine the seroprevalence of leptospirosis for the tea pickers, paddy workers, butchers and laughter house workers who is a risk group for leptospirosis in Turkey.

In this study, experimental group was consisted of 157 people, and the control group was consisted of 150 healthy individuals. The experimental group were occurred from tea pickers, paddy workers, butchers and laughter house workers who lives in Artvin and its counties. The control group were occurred of healthy individuals who hasn't a risk for leptospirosis in the same area. The presence of leptospirosis was determined by ELISA on serum samples. First step, 5µl serum sample was dropped into a box with capillary pipette on ELISA test kit. After, four drops of analysis dilute was added and test results were interpreted at 20 minutes in last step. Eleven *Leptospira* IgG/IgM (*Leptospira interrogans*) antibody test kit was used for this purpose.

The trial data were analyzed by SPSS 17.0. Number and percentage were used to determine the defining characteristics of the sampling and *t* test was used to search for differences between the groups. Leptospirosis was found positive of one person in the risk group of the study. Leptospirosis-positive people wasn't found in the control group. There were no significant differences in the presence of leptospirosis of between experimental and control groups.

According to the findings of the study that seropositivity of leptospirosis was found very low in the region.

**Keywords:** ELISA, Human, Leptospirosis

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### I. Introduction

Leptospirosis is one of the most common and important zoonotic infections in the world caused by *Leptospira* species spirochetes. *Leptospirae* are classified in two groups. *Leptospira interrogans* is the pathogen, and *Leptospira biflexa* is the saprophytic type. Leptospirosis is an infection caused by over 240 serotypes due to *Leptospira interrogans* in rodents, mammals, some birds and reptiles. *Leptospira interrogans* is a mandatory, aerobic, helicoidal, thin, motile microorganism with two periplasmic flagella. It is generally 6-20 µm long with a diameter of 0.1 µm. Its spirals are dense and fixed and one or both ends are curled as a hook, it has a spinning movement or moves sideways. It could be examined under dark field microscopy or by staining with special dyes [1].

Leptospirosis is an infectious disease induced by *leptospira* species in all domestic animals and humans and generally characterized by hemoglobinemia, icterohemoglobinuria, icterus, septicemia, and anemia [2, 3]. Leptospirosis is an important health problem among irrigated farm workers, freshwater fishermen, animal farmers, dairy and slaughterhouse workers, butchers and military staff in Turkey, similar to other countries [4, 5].

Primary mode of transmission in humans is the contact of water contaminated with the urine of infected animals and disintegrated skin-mucosa. Gastrointestinal tract transmission is also observed, albeit rare, by consumption of contaminated water and milk, and raw consumption of vegetables irrigated with contaminated water and contaminated meat. Transmission could also occur with inhalation of particles containing *Leptospira* or their contact with mucous membranes. It was reported that transmission could occur rarely as a result of mouse, dog or rat bites [6].

Leptospirosis is common in Black Sea Region in Turkey as reported generally in case reports or clinical series [7]. Regions with a wet climate, areas with several marshes and ponds and regions with a high rodent populations are more susceptible for the disease [8, 9].

Occupations such as farmers (especially paddy farm workers), slaughterhouse workers, miners, hunters, veterinarians, sailors and sewage workers are high risk groups for leptospirosis. In addition, the disease could be found among the practitioners of hobbies such as rafting, hunting and fishing or individuals who swim in lakes

and rivers. Optimum conditions for *Leptospirae* are warm, humid settings covered by neutral or slightly alkali waters. Seasons and occupation lead the factors that facilitate transmission. Leptospirosis is more common in rainy climates [9, 10, 11].

Artvin province is a Leptospirosis risk area due to its geographical location and seasonal properties. Especially rice in cultivation fields in Yusufeli township and tea cultivation areas in Hopa and Borçka townships, contamination risks are increased due to the humid and wet character of the land and possibility of contact with rodents. Furthermore, no comprehensive studies on leptospirosis were conducted in Artvin province. The objective of the present study is to conduct a serologic examination of leptospirosis among the risk groups including tea pickers, paddy farmers, butchers and slaughterhouse workers.

## II. Material And Method

**Study Time and Location:** The study was conducted between September 2012 and September 2013 in Artvin province townships.

**Sample Size:** The study was conducted with 157 volunteers living in Eastern Black Sea Region in Turkey at Artvin province townships and in occupations considered to have a risk for leptospirosis (tea pickers, paddy farmers, butchers and slaughterhouse workers) and 150 volunteers that lived in the same region, but were occupied in professions considered without leptospirosis risk.

**Collection of Blood Samples:** Study blood samples were collected during September 2012 when the seasonal rainfall was at the maximum level. Venous blood samples (5 ml) were collected from 157 risky, 150 healthy, a total of 207 individuals who volunteered for the study. Collected blood samples were coagulated at +4 degrees and then centrifuged at 3000 rpm in a cooled centrifuge to separate the serums. Serums were transferred into 2 ml Eppendorf tubes and stored at -81°C until use.

**Data Collection Tools:** Socio-demographic properties survey form was used as data collection tool to determine the demographic characteristics of the participants.

**Ethical Approval:** Before the data was collected, ethical committee approval was obtained for the study (B.30.2.ART.0.00.00.00/1143-04/06/2012). Then, the approval of the governor's office to conduct the study with the groups in provincial townships (B.10.0İSM.4.08.03.00.605-85/6247-11/09/2012) and informed consent from the participants were obtained.

**Serological Diagnosis:** ELISA test was conducted on obtained blood serums for serological examination.

**ELISA Test Kit:** For this purpose, 11 *Leptospira*IgG/IgM(*Leptospirainterrogans*)antibody test kits (SD, INC: *Leptospira*IgG/IgM, REF: 16FK40) were utilized. 1 test kit contains 30 separate packages of test kit, capillary pipette, analysis dilute, kit addenda.

**ELISA Method:** In the first step of ELISA protocol, 5 µl serum sample was dropped in S cartridge in the test kit using the capillary pipette. Then, 4 drops of analysis dilute was added and at the final step, test results were interpreted within 20 minutes.

**Data Analysis:** Statistical analysis were conducted with SPSS 17.0 software package on socio-demographic findings and serological analysis results.

## III. Findings

Certain demographic findings about the risk group with an average age of 51.82 are displayed in Table 1. Among the individuals in the risk group, it was identified that 45.2% were tea pickers, 33.1% paddy farmers and 21.7% were butchers and slaughterhouse workers. Majority of the risk group were females (74.5%) and 25.5% were males. Most of the individuals in risk group were primary school graduates (61.1%), while 17.8% were illiterate and only 2.8% were college graduates.

**Table 1: Risk group demographical findings**

	<b>Risk group</b>	<b>n</b>	<b>%</b>
<b>Profession</b>	Tea picker	71	45.2
	Paddy farmer	52	33.1
	Butchers and slaughterhouse workers	34	21.7
<b>Gender</b>	Female	117	74.5
	Male	40	25.5
<b>Education</b>	Illiterate	28	17.8
	Primary School	96	61.1
	Middle School	16	10.2
	High School	11	7.0
	College	6	2.8
<b>TOTAL</b>		157	100

Certain demographic findings about the control group with an average age of 21.03 are presented in Table 2. All individuals in the control group were high school graduates and 70.7% were female and 29.3% were male.

**Table 3: Control group demographical findings**

	Controlgroup	n	%
<b>Profession</b>	Student	150	100
<b>Gender</b>	Female	106	70.7
	Male	44	29.3
<b>Education</b>	High School	150	100
<b>TOTAL</b>		150	100

Distribution of *Leptospira* positive individuals in the risk and control groups is shown in Table 3. In the risk group, 0.6% of the participants were *Leptospira* positive, while the same rate was 0% in the control group.

**Table 3: *Leptospira* positive distribution in risk and control groups**

	Risk group		Controlgroup	
	Positive	Negative	Positive	Negative
<b>N</b>	1	156	0	150
<b>%</b>	0.6	99.4	0	100

Table 4 demonstrates the comparison of risk and control groups based on the incidence of leptospirosis. There was no statistically significant difference between the risk and control groups based on leptospirosis incidence.

**Table 4: Comparison of risk and control groups based on leptospirosis incidence**

Group	n	Std. Error	Df	Significance
Risk	157	0.080	305	t: 0.977
Control	150			p: 0.329

**Significance level  $p > 0.05$**

#### IV. Discussion

Although leptospirosis is defined as a zoonotic infection induced by *Leptospira interrogans* serotypes and characterized by jaundice, high fever and hemoglobinuria, it shows a complex clinical picture and is difficult to diagnose only with clinical pictures. It could be confused with diseases such as meningitis, typhoid fever, brucellosis, tuberculosis and pneumonia [12, 13].

It is quite difficult to culture and reproduce *Leptospirae* in artificial media and the method requires expertise. Thus, serological methods are preferred over others. The most frequently used serological tests are MAT and ELISA tests. MAT method is used almost in every country in *leptospira* diagnosis as an internationally accepted technique. Although it is a fast, specific and sensitive test, it has certain disadvantages such as a subjective assessment, open to human error, poor agglutination when the incubation period of the disease is longer and the arduousness of the method. On the other hand, ELISA is a fast, easy to apply, objective, and sensitive test and appropriate for scanning many serums and allows for specific IgG and IgM assays. Today, ELISA method, which is more sensitive and specific than MAT, is used in determination of *leptospira* IgM antibodies [6,7,10,13-18].

In the present study conducted to determine *leptospira* serotype prevalence among the risk groups of paddy farmers, tea pickers, butchers and slaughterhouse workers, 0.6% ELISA positive was found in the risk group, while no ELISA positive individual was identified in the control group (Table 3). There was no statistically significant difference between risk and control groups based on leptospirosis (Table 4). It was observed that leptospirosis prevalence was higher in other local and international studies.

Yarkin et al., in a study they conducted to determine leptospirosis prevalence and predominant *leptospira* serotypes in Çukurova region, determined 4.4% antibody response in the risk group [19]. Babür et al. analyzed the serums they obtained from 102 personnel working at Ankara province slaughterhouses to determine anti-leptospira antibodies using MAT and found seropositivity in two serums (1.96%) [6]. In another related study conducted in Turkey, Şencan et al. aimed to determine leptospirosis frequency among 279 healthy and 279 individuals with leptospirosis risk (farmers, veterinarians, paddy farmers) in Samsun province and assessed the collected serum samples using MAT. The results demonstrated that 4.3% of the risk group and 0.05% of the control group were seropositive [7].

Similar results were obtained in international studies as well. Benschop et al. conducted microscopic agglutination (MAT) test with 242 cattle slaughterhouse workers to research *leptospira* serotypes, *hardjo* and

*Pomonas* seroprevalence during the months of February and March and found the seroprevalence at 9.5% [20]. In a study Whitney et al. conducted with 511 veterinarian physicians in the USA, microscopic agglutination (MAT) test was conducted to determine the antibodies in physicians' blood serums against 6 *leptospira* serotypes and the results were 2.5% positive [21]. In a study conducted by Platts-Mills et al. in Peru, microscopic agglutination (MAT) test was conducted on blood samples of 250 randomly selected participants and 1.2% positivity was obtained [22]. In a study conducted with 166 professional soldiers and 216 civilian adult males in Australia, microscopic agglutination (MAT) test results demonstrated positive results in 23% of the participants [23].

It was observed that the results of both local and international studies reflected higher leptospirosis prevalence when compared to the results of the present study. The reason for the lower leptospirosis prevalence in this study seems to be the fact that individuals who are occupied with leptospirosis risk groups in Artvin province, who were the sample of the present study, utilized protective gear such as boots, overalls and protective goggles at work.

## V. Conclusion And Recommendations

In the present study conducted to determine leptospirosis seroprevalence among 157 volunteer individuals occupied in professions with leptospirosis risk (paddy farmers from Yusufeli township, slaughterhouse workers and butchers working at the provincial center and townships, tea pickers from Hopa and Borçka townships) living in Eastern Black Sea Region Artvin province townships and 150 volunteer individuals that were not engaged in occupations with leptospirosis risk, 0.6% ELISA positive was found in the risk group, while there was no ELISA positive individuals in the control group. However, there was no statistically significant difference between the risk and control groups based on leptospirosis prevalence.

As a result, it was determined that leptospirosis prevalence was lower in Artvin region when compared to the results of the studies conducted in other regions in Turkey. Thus, long-term serological studies are recommended in general population and occupational risk groups to determine regional differences and prevalence of leptospirosis.

## CONSENT

It is not applicable.

## COMPETING INTERESTS

Author has declared that no competing interest exist.

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