

# SEROLOGICAL STUDY FOR MEASURING RATE OF LEPTOSPIROSIS IN PATIENTS WHO ENCOUNTERED “SHALTOOK FEVER”

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**Abstract-** In Guilan province, near the end of spring and summer each year at the time of rice gathering, some of the farmers run high fevers. This disease is named as “Shaltook Fever” (Paddy or Husked rice fever) in the region. Review of literature showed outbreak of leptospirosis between rice farmers in Italy and Spain. We decided to measure antibody levels (IgM and IgG) against leptospirosis in patients with Shaltook fever. Samples were chosen from any patient who was diagnosed as Shaltook fever by health care clinics between end of spring and summer of 2002 in Guilan province. Patients should have fever above 38° C, with myalgia and history of recent work at rice fields. All other diagnosis for these patients should have been ruled out by clinical or laboratory investigations. Antibodies levels were measured by ELISA method. IgM was measured in 87 out of 100 serum samples and IgG was measured in 84 out of 87 of above serum samples. In 26 samples IgM anti *Leptospira* were positive (IgM > 20 u/ml), and 5 specimens were borderline (IgM = 15-20 u/ml). In measuring IgG, 10 samples were positive (IgG > 9 u/ml), and 13 samples were borderline (IgG = 5-9 u/ml). The above findings were suggestive of acute leptospirosis infection in 30% of Shaltook fever patients. It is of interest that in only 17 out of 26 positive samples for IgM positive IgG titers were observed. It could be concluded that these patients had, for the first time recently, encountered the disease.

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## INTRODUCTION

In 1886 Dr. Weil from Heidelberg, Germany, for the first time diagnosed the acute form of leptospirosis as an entirely separate entity from typhus, typhoid fever and hepatitis. This disease was later named after him as Weil syndrome. Thirty

years later, Inada *et al.* were able to separate the causative agent of disease. This bacteria was a spirochete from *Leptospira* family with two species, *L. biflexa* which is saprophyte and *L. interrogans* which is an infectious agent and up to now 200 different serotypes have been identified (1, 2). Further investigation showed that infection is widespread and could be identified in animals from pole to equator. The occurrence of disease is more frequent in humid areas because leptospira can survive for a long time in water and also transfer of disease is facilitated in these conditions (3-6).

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Leptospirosis is a zoonotic disease. The animal reservoir is very wide and consists of almost all mammals. *Leptospira* lives in kidney convoluted tubule, and in different subspecies it shows different duration for multiplication. With animals urine it contaminate water resources, if it's pH is not acidic. In the appropriate situation for the temperature, humidity and lack of any other contamination (bacterial or chemical) the bacteria could last for about 2 or 3 months and could be infectious for animals and human. Rodents and domestic animals are the most important reservoirs for infecting the humans (7-11).

Most often the disease is subclinical (*e.g.* in some places in equator 80% of population are serologically positive). About 90-95% of cases show minor symptoms such as fever and myalgia for a few days. In 30% of these cases a few days after subsiding of the symptoms the fever flare ups for immunological reasons. Sometimes aseptic meningitis and rarely uveitis accompanies the fever. Usually in these cases the diagnosis is confused with viral infections such as Enterovirus or Influenza. In 5-10% of cases the symptoms are severe and the patients show thrombocytopenia, renal failure and icter (Weil syndrome). Because of different presentation of the disease, in the passive monitoring system few cases could be find, but in an active mode of surveillance the number of the patient would raise significantly.

The first human case in Iran was reported by researchers in Razi Institute in 1960. He was a shepherd. In 1991 human Leptospirosis epidemic was reported in Guilan province (1, 8). Human leptospirosis was reported also from Mazandaran and Ghom Provinces. In Tehran, Markazi and Balouchestan provinces the domestic animals show a wide spread infection (7).

Near the end of spring and summer each year at the time of rice gathering, some of the farmers run high fevers. This disease is named as "Shaltook Fever" (Paddy or Husked rice fever) in the region. The possibility of another *Leptospira* epidemic and relationship between Shaltook fever and leptospira were the main reason for this research.

## MATERIALS AND METHODS

In this descriptive study the cases were chosen from patients who were either admitted in the hospitals or were visited in clinics of Guilan province from June till end of September of 2002. The chosen patients should have the symptoms of fever, myalgia, with or without conjunctivitis and icter and history of recent work at rice fields. All other diagnoses such as upper respiratory tract infection, urinary tract infection, gastroenteritis, typhoid and brucellosis should be ruled out and clinically they should have been diagnosed as Shaltook Fever. We obtained informed consent from all patients. After selecting the patient, a serum sample was obtained and freezed (-20°C). They were sent in safe box to Reference Laboratories of Iran-Research center for serological investigation in Buali Hospital, Tehran.

Serological investigations were conducted by ELISA method in serology department. The kit used was Serion/Virion (d-97072 Wurzburg Germany). All of the procedures were done according to the guidelines of the kit. Reference values were obtained from the kit and were IgM > 20 U/ml positive, 15-20 U/ml as borderline, < 15 U/ml as negative and IgG > 9 U/ml positive, 5-9 U/ml as borderline, < 5 U/ml negative.

We used SPSS-10 software for analysis of data.

## RESULTS

The results are shown in tables 1 and 2. Because of technical problems and bacterial and mycotic contamination, 87 out of 100 samples were measured for IgM and 84 of 87 of the above samples were measured for IgG. From the 87 samples 57 cases were male and 30 cases were females. And all of them were rice farmers or housewives, students or employees working part-time at rice fields. It is of interest that only 9 samples with positive IgM had positive IgG titer. One case had only positive IgG and 17 cases had only IgM and were encountered leptospirosis for the first time.

The result showed that 30% of Shaltook fever cases were in fact leptospirosis disease.

**Table 1.** IgM values for leptospirosis in Shaltook fever patients

	Frequency	Percent	Valid percent	Cumulative percent
<b>Ig M</b>				
Positive	26	29.9	29.9	29.9
Borderline	5	5.7	5.7	35.6
Negative	56	64.4	64.4	100.0
Total	87	100.0	100.0	
<b>Ig G</b>				
Positive	10	11.9	11.9	11.9
Borderline	13	15.5	15.5	27.4
Negative	61	72.6	72.6	100.0
Total	84	100.0	100.0	

## DISCUSSION

Guilan province has a history of *Leptospira* epidemic during the 90s of the last century and from then an increase in sporadic cases has been observed in Mazandaran and Qom provinces. Domestic animals are widely infected throughout the country (7, 8). In previous researches in Iran from 1976 to 1992 (7, 12-15) like other parts of the world the small mammals like mice and voles carries more

pathogenic serotypes such as icterohemorrhagica and grippityphosa (12,14) and cattle carry less virulent serotypes for men like Sejero and Hardjo (7).

Each year from end of spring to the end of summer a disease with fever and myalgia flares up in Guilan in rice farmers which is named Shaltook fever (Paddy fever). This study tried to show that this disease could be related to *Leptospira* infection.

One hundred serum samples were obtained from the diseased persons in the interval from June to September 2002. The *Leptospira* IgM and IgG were measures by ELISA method. A total of 26 people had positive IgM titers which accounted for 30% of cases; 17 out of 26 had only positive IgM titers (65%) which shows the first encounter with bacteria. Only 9 out of 26 had both IgM and IgG positive titers and one case had positive IgG only (Past infection). This finding shows spread of the disease between nonimmune populations who works in rice fields (farmers and part-time rice fields workers). To find the cause of the disease in the rest 70%, they should be tested for viruses that can cause seasonal fever (enteroviruses and adenovirus), and other common diseases with fever in the region such as typhoid and brucellosis, and even Hanta virus though it has not been reported in the country yet (2).

In 5% of cases where icter accompanies other symptoms (Weil Syndrome), the differential diagnosis should include fulminant hepatitis, septicemia (staphylococcal, meningococcal), Crimean Congo hemorrhagic fever and thrombotic thrombocytopenic purpura.

**Table 2.** Descriptive study of IgM and IgG values in shaltook fever patients

		statistic	Std. Error
<b>IgM</b>	Mean	26.682	3.5191
	95% confidence interval for mean	19.683	
	lower bound		
	Upper bound	22.681	
	Median	10.400	
	Std. Deviation	32.2531	
	Minimum	3.0	
	Maximum	100.0	
<b>IgG</b>	Mean	6.581	1.0099
	95% confidence interval for mean	4.572	
	lower bound		
	Upper bound	8.590	
	Median	4.000	
	Std. Deviation	9.2562	
	Minimum	1.1	
	Maximum	56.0	

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## Conflicts of Interests

We have no conflicts of interest.

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