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# Serodiagnosis of human leptospirosis by Enzyme Linked Immunosorbent Assay (ELISA)

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## RESEARCH

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## ABSTRACT

**Background**
Leptospirosis is a zoonotic bacterial disease that affects humans and animals. The disease occurs by contact direct or indirect with the urine of infected animal and common among agriculture workers, garbage collectors, sewage works, forestry and animal slaughtering. The disease also spreads in tropical regions where the conditions are favourable for leptospires.

**Aims**
The purpose of this study is to determine human leptospirosis among suspected cases by ELISA IgM (a retrospective study on 50 cases from 2004 to 2010).

**Methods**
Sera from patients had fever and jaundice suspected clinically from leptospirosis, were referred to the National Institute of Hygiene in Rabat, Morocco. ELISA IgM and SAT were used for the diagnosis.

**Results**
While 33 serums were positive by Slide Agglutination Test (SAT), thirty one serums were positive by ELISA IgM. The sensitivity was 62 per cent and 66 per cent in ELISA and SAT respectively.

**Conclusion**
ELISA IgM and SAT seem to be useful for human leptospirosis; they can detect the disease from the 5th day of the illness. They are easy, inexpensive and useful for developing countries and less equipped laboratories. However, if the first sera was negative, the second sera should obtained in the second week, or PCR used in combination with serological tests if it is available.

**Key Words**
Leptospirosis, SAT, Slide Agglutination Test, ELISA, Morocco, Zoonosis.

## Implications for Practice:

**1. What is known about this subject?**
Leptospirosis is an infectious disease, which affects human and animal. It is underreported due to nonspecific symptoms, challenging diagnostics and poor access to health care.

**2. What new information is offered in this case study?**
One serum at the acute phase of leptospirosis is not enough for diagnosis by ELISA and SAT, the second serum at the convalescent phase (second week) should be taken, or PCR should be used in combination with serological tests.

**3. What are the implications for research, policy, or practice?**

Early diagnosis is essential because of the risk of severe complications in the absence of early antibiotic treatment including pancreatitis, lung and intracranial haemorrhages.

## Background

Leptospirosis is a zoonotic bacterial disease that affects vulnerable population such as rural subsistence farmers and urban slum dwellers. Although leptospirosis causes life threatening clinical manifestations, such as pulmonary hemorrhage syndrome, and has a worldwide distribution, the key barrier to addressing this neglected disease has been insufficient data on its disease burden.[1] The disease is considered an occupational disease, many cases having been observed among persons engaged in agriculture, sewage works, forestry, and animal slaughtering;[2] agriculture workers and garbage collectors are amongst persons the most at risk.[2,3] Global climate change (flooding, heavy rainfall and cyclones) has resulted in an upsurge in the disease incidence and outbreaks.[4] According to the World Health Organization (WHO,2003), the incidence of the disease range from 0.1–1 per 100,000 per year in the temperate regions, 10–100 per 100,000 in the humid tropics and the incidence may reach over 100 per 100,000 during outbreaks.[5] Diagnosis of leptospirosis depends on the phase of the infection; leptospires usually circulate in the blood of the patient for about 10 days after the onset of the disease. Detectable titres of antibodies appear in the blood about 5–10 days after the onset of disease, but sometimes later, especially if antibiotic treatment is instituted.[5] Generally, during the first week after the onset of symptoms, leptospirosis is diagnosed by PCR amplification of bacterial DNA from the blood, or by detection of antibodies during the second week of the disease.[6] PCR is demonstrably useful for early diagnosis, but it is unavailable in most developing countries. Early diagnosis is essential because antibiotic treatment is most effective when initiated early in the course of the disease.[7] Developing countries need low cost and effective diagnosis such as ELISA IgM which is used for early diagnosis of leptospirosis during the acute phase, in humans,[8] and animals.[9] Slide Agglutination Test( SAT) is another method commonly used for early detection of leptospira antibodies, the technique is sensitive and easy to use for early diagnosis of leptospirosis.[10] In this study, ELISA IgM and SAT were evaluated for the diagnosis of human leptospirosis.

## Methods

Patients and serum samples: During 2004 to 2010, fifty sera specimens from 50 patients were referred to the National Institute of Hygiene, Rabat, Morocco. A total of 50 sera included in this study; 1(2 per cent) was from Beni Melal, 21(42 per cent) were from Meknes, 1(2 per cent) was from Agadir, 2 (4 per cent) were from Rabat, 2 (4 per cent) were from Taza, 2 (4 per cent) were from Salé, 2 (4 per cent) were from Tanger, 9 (18 per cent) were from El Jadidah and 10 (20 per cent) were from Sidi Kacem. Single sera was used and the results of ELISA IgM in this study were compared with the results of SAT conducted previously.[11] Clinicians suspected with leptospirosis due to some sings occurred mimic this disease, particularly fever and jaundice. Sera were referred to the laboratory of bacteriology in the National Institute of Hygiene in Rabat for diagnosis however, other information such as epidemiology conditions, occupations and the date of sampling were not indicated in all sera.

ELISA IgM: Detection of IgM antibodies was determined using a commercially available leptospira IgM ELISA kit from nal von minden, Germany. Sera and controls were diluted 1:100 and performed according to the manufacturer's instructions. Each sera had an absorbance ratio greater than that of the cut-off calibrator was defined as positive (results in Units [U] = Patient (mean) absorbance value$_x$10/Cut-off). The result considered positive if U›11, negative if U‹9 and if U between 9–11 it is recommended to repeat the test with a fresh sample in 2–4 weeks.

## Results

While 33 sera were positive by Slide Agglutination Test (SAT), thirty one sera were positive by ELISA IgM, (table1). According to the cases distribution, the total number of cases from 2004–2010 were 23, 5, 1, 0, 2, 8 and 11 respectively, (table2).

## Discussion

Limitation of ELISA for the diagnostic of leptospirosis was observed.[12] IgM class antibodies may remain detectable for several months or even years particularly in endemic areas, as well as some ELISA test systems are less specific than the MAT and weak cross reactions due to the presence of other diseases may be observed. Therefore, ELISA results should be confirmed by MAT the gold serological test for leptospirosis,[5] or testing a second sample at the convalescent phase for seroconversion or a significant rise in titre should be required in cases with an initial negative result.[13]

Although all limitation, ELISA IgM can be used in the first week and its sensitivity seems to be higher than the MAT

(the international standard). However, the sensitivity will be increased in the second week (table 3) when the IgM antibodies have had time to develop.[14] Usually, nonpathogenic *Leptospira biflexa* serovar Patoc (strain Patoc I) used as antigen. It has been indicated that sera from patients with leptospirosis cross-reacted with antigens from this nonpathogenic strain.[7] However, antigens isolated from one or preferably multiple locally dominant pathogenic species, could improve sensitivity and specificity of the assay instead of the standard bacterial isolates.[15] Another ELISA contained mixture of some antigens was used by ANGELA P. BRANDAO et al.[14] who indicated that the sensitivity of SAT and ELISA IgM in the first week were 57 per cent and 53 per cent respectively, comparing with 34 per cent for MAT the gold standard serodiagnostic method. While in the second week, the sensitivity for both SAT and ELISA IgM were 83 per cent. Due to this study is a retrospective study, we cannot obtain another sera, as well as the time of sampling was not considered by the clinicians from the past and at the time of this article.

SAT is easy, safe, inexpensive, very convenient for developing countries and less equipped laboratories,[8,10,12,14] and can be used for human and animal leptospirosis.[16] Although, sensitivity of SAT seems to be more than ELISA IgM at the acute phase,[10,14] PCR was recommended to use in combination with serological tests.[17] PCR can detect leptospirosis from blood samples during the first week and had more sensitivity and considered that PCR based diagnosis of leptospirosis should be made available for clinicians for the early diagnosis and prompt treatment of the disease.[18] According to our results, the highest incidence was in 2004, due to leptospirosis outbreak occurred in Meknes region, seventy seven cases of human leptospirosis were declared by the Ministry of Health,[19,20] out of 77 sera declared 23 were referred to the laboratory of the National Institute of Hygiene in Rabat. All of eight cases reported in 2009 were from El Jadidah, Haraji, 2011 indicated an outbreak occurred in 2009 in this region.[21] Of 11 cases in 2010, ten of them were from Sidi Kacem and only one sera was from El Jadidah. High incidence in Sidi Kacem was reported, however the epidemiological information is unavailable.[22]

## Conclusion

Leptospirosis may become positives by serological tests after the 5th day, at this stage the diagnosis can be done according to clinical and epidemiological criteria or PCR if it is available. PCR is expensive and unavailable in developing countries where the most cases are. ELISA and SAT can be used for human leptospirosis; these techniques can measure IgM antibodies and become positive by the 5th day. They seem to be useful for current infection and require one sample comparing with MAT, which need two samples for the diagnosis. However, the second sample must be obtained if the first was negative and clinical signs occurred. In this study, although clinical manifestation was observed, poor positivity was noted for ELISA IgM and SAT, might due to one serum used or other factors such as patients may treated before the raise of antibodies or they were under medication. We strongly recommend the clinicians, if the first serum is negative by serological tests (ELISA IgM and SAT), the second serum in the second week of the illness should be obtained from the patient, or conduct the diagnosis in combination with PCR and serological tests.

## Limitations

1. No availability to compare our results with gold serological test (MAT).
2. No possibility to obtain the second serum at the convalescent phase.
3. Date of sampling was not indicated by clinicians.
4. Epidemiology factors and occupations were not considered in all sera referred.

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## PEER REVIEW

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## CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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None

**Table 1: Results and the positivity of ELISA IgM and SAT**

| The test | Positive | Negative | Positivity |
|---|---|---|---|
| ELISA IgM | 31 | 19 | 62% |
| SAT | 33 | 17 | 66% |

**Table 2: The total number of cases during 2004 to 2010**

| 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 |
|---|---|---|---|---|---|---|
| 23 | 5 | 1 | 0 | 2 | 8 | 11 |

**Table 3: Sensitivity of ELISA IgM and SAT according to other studies**

| Duration of illness in days | Sensitivity | | References |
|---|---|---|---|
| | ELISA % | SAT % | |
| 2-3 | 28 | ND | 19 |
| 4-5 | 54 | | |
| 6-7 | 77.8 | | |
| 0-6 | 53 | 57 | 14 |
| 7-14 | 83 | 83 | |
| 15-60 | 99 | 99 | |
| 4-5mo | 92 | 50 | |
| 6-8mo | 74 | 17 | |
| ND | 63.6 | 91 | 11 |
| 3-8 | 79.3 | 72.4 | 17 |
| 9-14 | 100 | 100 | |
| >14 | 88.8 | 77.7 | |
| 02-7 | 62.1 | ND | |
| 8-13 | 91.7 | | 8 |
| 14-19 | 100 | | |

ND: Not determined