

Diagnosis of Human Leptospirosis in Morocco by IgM ELISA and Slide agglutination test (SAT)

Waleed Al-orry^{1,2}, M. Arahou¹, R. Hassikou¹, A. Quasmaoui², R. Charof², and Z. Mennane²

¹Laboratory of Botany, Department of Biology, Faculty of Sciences, Rabat, Morocco

²Department of Medical Bacteriology, National Institute of Hygiene, Ibn Battuta Avenue, B.P. 769, Agdal, Rabat 11000, Morocco

Copyright © 2016 ISSR Journals. This is an open access article distributed under the *Creative Commons Attribution License*, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT: Serology plays an important role in the diagnosis of leptospirosis. Few laboratories have the resources and expertise to perform the microscopic agglutination test and leptospirosis remains a neglected disease because of limited access to diagnosis, due to reliance on antiquated standard methods and the cost of commercially available alternatives. There is a need for rapid and simple serological tests which facilitate the early diagnosis of leptospirosis, while antibiotic therapy may be most effective. In Morocco this disease is little known. Studies about it are very rare. In this study 11 serums which referred to the National Institute of Health in Rabat, Morocco during 1-1-2014 to 30-6-2015 were evaluated by IgM ELISA and Slide agglutination test (SAT). 7 serums were positives by Elisa and 10 were positives by (SAT). 9 of cases were from Sidi kecem region. All patients were male. The rate of age for all patients was 29.5 years.

KEYWORDS: leptospirosis, Morocco, Elisa, SAT, Slide agglutination test.

1 INTRODUCTION

Leptospirosis is an acute bacterial infection caused by spirochetes belonging to the genus *Leptospira* that can lead to multiple organ involvement and fatal complications. It has a wide geographical distribution and occurs in tropical, subtropical and temperate climatic zones with mortality more than 500,000 cases are estimated to occur worldwide each year [1],[2]. Leptospirosis is maintained by the persistent colonization of the renal tubules of carrier animals, and it appears that almost all mammals are susceptible to be natural carriers of *Leptospira* [3]. An infected animal can remain symptom-free and shed infectious organisms in its urine, either transitorily or for its entire lifetime, humans can be infected directly by contact with the urine of an infected animal or indirectly from the contaminated environment [4]. The genus *Leptospira* comprised the saprophytic subgroup (with six known species), the pathogenic subgroup (nine species), and the intermediate subgroup (five species) the pathogenicity of which remains unclear (5). The pathogenic species comprise more than 250 serovars belonging to approximately 24 serogroups based on agglutinating lipopolysaccharide antigens [5]. The clinical presentation of leptospirosis in humans is difficult to distinguish from dengue, malaria, influenza, and many other diseases characterized by fever, headache, and myalgia [4]. Leptospirosis produces a spectrum of clinical manifestations which range from a mild febrile illness to severe disease forms such as Weil's syndrome and severe pulmonary hemorrhage syndrome, and the case fatality rate for severe forms of leptospirosis is 5 to 40%, respectively [6]. Diagnosis of leptospirosis is based on laboratory confirmations because its clinical signs are nonspecific and may be mistaken with other febrile diseases [7]. *Leptospira* are present in the blood during the first week of infective symptoms. Culture is rarely performed in routine clinical practice since this may take several months and requires considerable expertise [4]. Antileptospire IgM may be detected 4 to 5 days after the onset of symptoms, before detection of IgG and of agglutinating antibodies, and persist at least 5 months in patients [8],[9]. Conventional serological methods such as enzyme-linked immunosorbent assay (Elisa) are widely used for the diagnosis of leptospirosis and several authors have reported that antileptospire antibodies could be detected earlier with this test than with the MAT [4],[9]. IgM ELISA is promising because it can be performed in a greater number of laboratories

throughout the tropics, and is inexpensive compared with MAT. The IgM ELISA has been recommended by the World Health Organization (WHO) as a diagnostic test for the serodiagnosis of leptospirosis where healthcare resources are limited [3]. although its reported accuracy is variable. A number of studies have reported that IgM ELISA has high sensitivity and specificity for the diagnosis of acute leptospirosis [10],[11]. The slide agglutination test (SAT) is an inexpensive assay which can be performed quickly and easily [12]. some authors compared IgM-ELISA and SAT with MAT. They demonstrated that the overall results for antibody detection by those three assays were similar. However, the SAT and ELISA were statistically more sensitive as initial screening test [13]. IgM-ELISA may be used for the diagnosis of leptospirosis. However, the agglutination test is useful for screening and for secondary infection cases for which IgM antibodies may be undetectable [13]. It is a highly sensitive serologic technique which has been used to aid the diagnosis of many bacterial, viral and parasitic diseases [4].

2 MATERIALS AND METHODS

2.1 PATIENTS AND SERUMS SAMPLES

All blood samples for this study were referred to the National Institute of Health in Rabat, Morocco. Between the year 2014 and .6.2015 , the laboratory received from certain regions in morocco ,11 samples for confirmation and identification. Samples were centrifuged, and the serum was collected and stored at -20°C until it was assayed.

2.2 LEPTOSPIRA IGM ELISA

Detection of IgM antibodies to *Leptospira* species was determined using a commercially available *Leptospira* IgM ELISA from nal von minden. The assay was performed according to the manufacturer's instructions. Sera were diluted 1:100 in serum diluent, and 100 µL from cutoff, and positive and negative control were added to *Leptospira* antigen-coated microwells and incubated for 60 minutes at 37°C. When incubation has been completed, remove the foil and wash each well three times with 300 µL of Washing Solution. Dispense 100 µL of *Leptospira* anti-IgM Conjugate into all wells except for the Substrate Blank well and incubate for 30 minutes at room temperature. Dispense 100 µL of TMB Substrate Solution into all wells and incubate for 15 minutes at room temperature. Dispense 100 µL of Stop Solution into all wells and measure the absorbance of the specimen at 450/620 nm within 30 minutes after addition of Stop Solution. The recommended cutoff for a positive result is more than 11 units.

2.3 SLIDE AGGLUTINATION TEST (SAT)

Leptospira antigen purchased from Bio-Rad (Marnes-la-Coquette, France) was mixed with two-fold of serum. The agglutination was observed.

3 RESULTS AND DISCUSSION

A total of 11 serum specimens from 11 patients diagnosed with leptospirosis referred to the National Institute of Health in Rabat Morocco were examined. All patients were suffered from jaundice and fever. 9 patients were from Sidi kecem region, one was from Jarf El Melha and one from Belkasiri. The age was (14-53) years. All patients were males. 10 serums were positives with agglutination test and only 7 were positives with IgM Elisa. According to IgM Elisa procedure we considered the results positives which had titers > 11 units, titers equivocal which had 9-11 and titers which had <11 unites considered as negatives. The titers positives and the negatives were described in Table 1. One case was in February, two in March, five in April, two in May and one in June.

Table 1: results of IgM Elisa and Agglutination Test

Serums	1	2	3	4	5	6	7	8	9	10	11
IgM Elisa	13U +	13.5U +	23U +	8.5U -	18U +	21U +	5U -	11U +	7.5U -	27U +	1U -
SAT	+	+	+	+	+	+	+	+	+	+	-

In our study, we found that all patients were men and this is consistent with other authors who reported that men infected with leptospirosis are more than women [14].

The IgM-ELISA used in this study couldn't detect antibodies in all cases, because either Sampling was in the early days before the appearance of antibodies or by the presence of other diseases. Alan J. A et al. [15] indicated the sensitivity of Elisa was low (62.1%) during the first week of illness. Of note, sensitivity increased to 91.7% during the second week. Therefore, testing of a late acute-phase sample (≥ 10 th day of illness) is recommended when an initial sample is negative. In this study we can't obtain another serum to perform the second diagnoses by IgM Elisa. Leptospirosis which was diagnosed by IgM Elisa and SAT by Chintana Chirathaworn et al. [13] demonstrated some results were negative by IgM Elisa when the first samples were tested despite the clinical findings are suggestive or the agglutination test is positive. This confirms that when the result of the IgM-ELISA for the first sample is negative but the clinical findings are suggestive or the agglutination test is positive, the second sample for IgM-ELISA testing should be examined. On the other hand Bajani MD et al. [16] reported that negatives results may occurs by the presence of antibodies due to previous exposure (in endemic regions), and by the presence of other diseases, this means specificity of IgM detection by ELISA is affected by the antigen used in the assay. It is a suitable screening test for the determination of *Leptospira* IgM antibodies in the routine clinical laboratory [17]. The ELISA showed excellent sensitivity (100%) and high specificity (93%) in the diagnosis of acute *Leptospira* infection, as reported in other studies [18].

In Morocco this disease is little studied. M.HARAJI et al. [19] found in El Jadida Maroc one patient, 22 years old, was admitted to Mohamed V hospital in El Jadida presenting clinical symptoms assimilable with leptospirosis. Mohamed ANOUAR SADAT [20] demonstrated that poultry market workers of Casablanca were at substantial risk of exposition to leptospirosis because of bad hygienic conditions and presence of rodents in both their workplace and their place of residence.

We found that majority of cases were between February and May, this months are the months of rainfall in Morocco. Yanagihara Y et al [21] demonstrated that Significant exposure occurs from normal daily activities, with high rates of infection during heavy rainfall and flooding.

In Mumbai, India, an eight-fold increase in disease incidence was noted after severe flooding in 2005. In Manila, Philippines, a large outbreak of leptospirosis was reported after tropical storms and severe flooding in October 2009. A higher seroprevalence of infection has also been associated with heavy rainfall and flooding in China, France, Brazil, Trinidad and Tobago, and French Polynesia [22].

In this study all cases were males, this consists with ALAN R. KATZ et al. [23] who found in a study conducted in America that cases were predominately male. Sidi kacem is a rural region in Morocco, ALAN R. KATZ et al.[23] also indicated rates were highest in rural areas.

4 CONCLUSION

1. Studies in Morocco about Leptospirosis is very rare.
2. From 11 serums referred to the National Institute of Health in Rabat, Morocco during 1-1-2014 to 30-6-2015. 7 were positives by Elisa and 10 were positives by (SAT).
3. Elisa is more sensitive than SAT but in Leptospirosis another serum is very important to diagnostic this disease.
4. In this study we can't obtain another serum. So in this case SAT results may be correct more than Elisa.
5. The majority of injuries were during the rainy season, therefore we believe that rainfall helped incidence.
6. We think that Sidi kacem region is an affected area in Morocco by Leptospirosis, It may be likely that there are many cases of unknown.
7. We recommend the need to do more studies in this area. As well as using the methods of prevention and vaccination against this disease.

ACKNOWLEDGEMENTS

I thank all colleagues and staff in the laboratory of microbiology medical at the National Institute of Health in Rabat on the cooperation and support.

REFERENCES

- [1] Waitkins SA., Leptospirosis. In: Manson's Tropical Disease, 19th edn. Eds. Manson-Bhor PEC, Bell DR. London, Baillière Tindall. 19th edn. P657-665,1987.
- [2] World Health Organization: Leptospirosis worldwide, *Wkly Epidemiol Rec*, 74:237–242,1999.
- [3] World Health Organization: Human leptospirosis, guidance for diagnosis, surveillance and control. Malta: World health Organization; 2003.
- [4] P.N. Levett, "Leptospirosis", *Clin Microbiol Rev*, vol.14, pp. 296–326,2001
- [5] Ko.I. Goarant C., Picardeau M., "Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen". *Nat Rev Microbiol*, vol. 7, no.10, pp. 736–747, 2009.
- [6] Faine S, Adler B., Bolin C., Perolat P., *Leptospira and Leptospirosis*. ISBN 0 9586326 0 X. 2 ed. Melbourne, Australia, MediSci, 1999.
- [7] L. Vado-Solis, M.F. Cardenas-Marrufo, B. Jimwnez- Delgadillo, A. Alzina- Lopez, H. Laviada-Molina, V. Suarez-Solis, J.E. Zavala-Velazquez, "Clinical-epidemiological study of leptospirosis in humans and reservoirs in Yucatan, Mexico", *Rev Inst Med Trop*. Vol. 44, no. 6, pp. 335-340, 2002.
- [8] M.V. Silva, E.D. Camargo, L. Batista , A.J. Vaz , A.P. Brandão , P.M. Nakamura, et al., "Behaviour of specific IgM, IgG and IgA class antibodies in human leptospirosis during the acute phase of the disease and during convalescence", *J Trop Med Hyg*, vol. 98, pp. 268–72,1995.
- [9] M. Picardeau, "Diagnosis and epidemiology of leptospirosis", *Médecine et maladies infectieuses* vol.43, pp. 1-9, 2003.
- [10] M.C. Ooteman, A.R. Vago, M.C. Koury, " Evaluation of MAT, IgM ELISA and PCR methods for the diagnosis of human leptospirosis", *J Microbiol Methods*, Vol. 65, pp 247-257, 2006.
- [11] W.J. Zochowski W, M.F. Palmer, T.J. Coleman," An evaluation of three commercial kits for use as screening methods for the detection of leptospiral antibodies in the UK", *J. Clin Pathol*, vol. 54 pp. 25-30, 2001.
- [12] B. Adler, A.M. Murphy, S.A. Locarnini, S. Faine, "Detection of specific antileptospiral immunoglobulins M and G in human serum by solid-phase enzyme-linked immunosorbent assay ", *J. Clin. Microbiol*, vol. 11, pp. 452-457, 1980.
- [13] Chintana Chirathaworn et al., "COMPARISON OF A SLIDE AGGLUTINATION TEST, LEPTOTEK DRI-DOT, AND IGM-ELISA WITH MICROSCOPIC AGGLUTINATION TEST FOR LEPTOSPIRA ANTIBODY DETECTION", *SOUTHEAST ASIAN J TROP MED PUBLIC HEALTH*. Vol. 38, no. 6, pp. 1111-1114, 2007.
- [14] Mlle LAMRANI ALAOUI Ghita, THESE, UNIVERSITE SIDI MOHAMMED BEN ABDELLAH FACULTE DE MEDECINE ET DE PHARMACIE, Fès, Maroc, (2008).
- [15] J.A. Alan, A. McBride Fernanda, D. Pereira Emilson, B. da Silva Rosan, D. de Matos Edimilson, G. da Silva Antônio, P. Ferreira Mitermayer, I.K.O. Albert," Evaluation of the EIE-IgM Leptospire. assay for the serodiagnosis of leptospirosis", *Acta Trop*, vol. 102, no. 3, pp. 206–211, 2007.
- [16] M.D. Bajani, D.A. Ashford, S.L. Bragg, C.W. Woods, T. Aye, R.A. Spiegel, B.D. Plikaytis, B.A. Perkins, M. Phelan, P.N. Levett, R.S. Weyant, " Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis", *J.Clin Microbiol*. Vol. 41, pp. 803-809, 2003.
- [17] E. WILLIAM, J. WINSLOW DAVID, L. MERRY MOIRA, L. PIRC, L. PETER, " Evaluation of a Commercial Enzyme-Linked Immunosorbent Assay for Detection of Immunoglobulin M Antibody in Diagnosis of Human Leptospiral Infection ", *JOURNAL OF CLINICAL MICROBIOLOGY*. Vol. 35, pp. 1938-1942, 1997.
- [18] M.V. Da Silva, A.J. Camargo, L. Batista, " Immunodiagnosis of human leptospirosis using saliva", *Trans. R. Soc*, Vol. 86, no. 5, pp. 560-561, 1992.
- [19] M. HARAJI, et al.," . Forme ictérique de la Leptospirose Humaine: présentation d'un cas à El Jadida, Maroc", *LES TECHNOLOGIES DE LABORATOIRE*, vol. 6, no.23, pp. 36-41, 2011.
- [20] Mohamed ANOUAR SADAT," SEROPREVALENCE DE LA LEPTOSPIROSE CHEZ LES PROFESSIONNELS A RISQUE DE LA VILLE DE CASABLANCA", *Mémoire de fin d'études* . (2014).
- [21] Y. Yanagihara, Villanueva SYAM, S. Yoshida, Y. Okamoto, T. Masuzawa," Current status of leptospirosis in Japan and Philippines" , *Comp Immunol Microbiol Infect Dis*, vol. 30, pp 399-413, 2007.
- [22] L. Colleen, Lau et al.," Climate change, flooding, urbanisation and leptospirosis: fuelling the fire?", *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 104, pp. 631-638, 2010.
- [23] R. ALAN, KATZ et al.," LEPTOSPIROSIS IN HAWAII, 1974–1998: EPIDEMIOLOGIC ANALYSIS OF 353 LABORATORY-CONFIRMED CASES", *J. Trop. Med. Hyg*, vol. 66, no.1 pp. 61- 70, 2002.