

Human Leptospirosis in Morocco, 2004-2010: Serological Study by Slide Agglutination Test (SAT)

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ABSTRACT—*Leptospirosis is an emerging zoonosis with a worldwide distribution but is more commonly found in impoverished populations in developing countries and tropical regions with frequent flooding. The rapid detection of leptospirosis is a critical step to effectively manage the disease and to control outbreaks in both human and animal populations. Culture and the microscopic agglutination test are gold standard methods for leptospirosis diagnosis; however, they are not useful for early diagnosis. PCR is demonstrably useful for early diagnosis, but it is unavailable in most developing countries. Therefore, there is a need for accurate and rapid diagnostic tests and appropriate surveillance and alert systems to identify outbreaks. In this study 50 serums of suspected cases with leptospirosis were referred from certain regions in Morocco, to the National Institute of Health in Rabat, Morocco, during 2004 to 2010, Slide Agglutination test (SAT) was used for confirmation. Total number of cases were 23,5,1,0,2,8 and 11. SAT positive results were 20,4,0,0,1,6 and 2, in 2004,2005,2006,2007 ,2008,2009,2010 respectively. The number of males was more than females (31 males and 19 females). The serums were from different regions (BeniMelal, Meknas, Agadir, Rabat, Taza, Salé, Tanger, El Jadidah and Sidi Kacem).*

Keywords---Leptospirosis, Morocco, SAT, Zoonosis

1. INTRODUCTION

Leptospirosis is emerging as an important public health problem across the world, It is a zoonotic disease and a wide variety of wild and domestic animals have been implicated as carriers. People get infected accidentally when they come into contact with an environment contaminated with *Leptospira* or, less frequently, tissue, body fluids or urine of carrier animals. The disease was originally described more than a century ago as Weil's disease, which is characterized by acute febrile illness with icterus, splenomegaly and nephritis (Vijayachari et al.,2008; Haake and Levett,2015). More than 1.7 million cases of severe leptospirosis are reported each year, with case mortality rate about 10%. It is most common in developing countries, particularly in the Caribbean, Latin America, the Indian subcontinent, Southeast Asia, and Oceania, although locally acquired cases in industrialized countries are well described (Chen et al.,2013). It has been recognized as an important occupational hazard of agriculture manual laborers, sewage workers, animal handlers, forestry workers and other outdoor workers who work in wet conditions, and butchers (Vimala et al.,2014). Symptoms of leptospirosis are nonspecific and may be easily confused with other febrile illnesses, such as dengue or malaria, which require different treatment regimens (Chen et al.,2013) therefore misdiagnosis is a major problem in regions where other causes of undifferentiated febrile illness and hemorrhagic fever are endemic. Timely diagnosis relies on an effective laboratory test, since the presentation of early-phase leptospirosis is often nonspecific (McBride et al.,2007). Although The microscopic agglutination test (MAT) is the reference serological assay, MAT is a laborious and time consuming technique (Chirathaworn et al 2007). As well as it showed low sensitivity in the early days of illness, as has also been reported from other studies. It remains very useful for epidemiologic studies, identification of strains, assessment of the probable infecting serogroup, and confirmation of illness for public health surveillance. While Slide Agglutination test (SAT) seems to be a convenient test for the initial diagnosis of leptospirosis (Brandão et al.,1998), and presenting high sensitivity in the acute phase (Chirathaworn et al 2007). SAT is inexpensive, can be performed more quickly and more easily than ELISA and MAT and could be used by the less well equipped laboratories. Therefore Early detection demands a rapid and sensitive diagnostic test, because of limited sensitivity in the early stage of the disease (Brandão et al.,1998). SAT technique was developed in 1958 by Galton (Galton et al.,1958). Stoenner and Davis have modified the

preparation of plate antigens for leptospirosis diagnostic and concluded that this antigen could be used in rapid tests, obtaining similar sensitivities with the MAT in human, porcine, bovine sera (Stoenner and Davis, 1967). The main disadvantages of SAT are that it is not suitable for epidemiological studies, identification of strains, assessment of the probable infecting serogroup, and confirmation of illness for public health surveillance. Interpretation may be doubtful and it shall be used only in acute cases of clinical leptospirosis, mainly in endemic areas (Lilenbaum et al., 2002). In Morocco this disease is little known, it was raised for the first time by Melnotte and Farjot who reported 7 cases of hemorrhagic spirochaetosis ictero-in Fez in 1927. During 1950 and 1962 the maximum work and research on leptospirosis were conducted under the direction of Dr. White with the participation of Mailloux and Kolochine Erber (Rais, 1997). In 2011 HARAJI (HARAJI et al., 2011) 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 and SADAT (SADAT, 2014) 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. Recently we conducted a study by Elisa and SAT on 11 serums collected during 1-1-2014 to 30-6-2015, ten of them were positive by SAT (Al-orry et al., 2016).

2. MATERIAL AND METHODS

2.1. Serums samples

All blood samples for this study were referred to the National Institute of Health in Rabat, Morocco, during 2004 to 2010, the laboratory of microbiology received from certain regions in Morocco, 50 samples for confirmation and identification. Samples were centrifuged, and the serum was collected and stored at -20°C until it was assayed.

2.2. Slide Agglutination test (SAT)

Leptospira antigen purchased from Bio-Rad (Marnes-la-Coquette, France) was used. Tests were performed as previously described (Lilenbaum et al., 2002). Antigenic suspension was homogenized immediately after the use. Volumes of 15 µl of each undiluted serum to be tested were added to 55 µl of antigenic suspension on a glass slide, and mixed with sticks, and placed 4 minutes in room temperature. The agglutination was observed under direct light.

3. RESULTS AND DISCUSSION

The results of Leptospira antibody detection are shown in Table 1. Fifty serums during 2004-2010 from 50 patients suspected with leptospirosis referred to the National Institute of Health in Rabat Morocco were diagnosed by Slide Agglutination test (SAT). All patients were suffered from fever and majority of them had jaundice. Total numbers of cases were 23, 5, 1, 0, 2, 8 and 11. SAT positive results were 20, 4, 0, 0, 1, 6 and 2, in 2004, 2005, 2006, 2007, 2008, 2009, and 2010 respectively. The number of males was more than females (31 males and 19 females). The serums were from different regions (Beni Melal, Meknas, Agadir, Rabat, Taza, Salé, Tanger, El Jadidah and Sidi Kacem), majority of cases (21 serums) were from Meknas (Table and Figure 2).

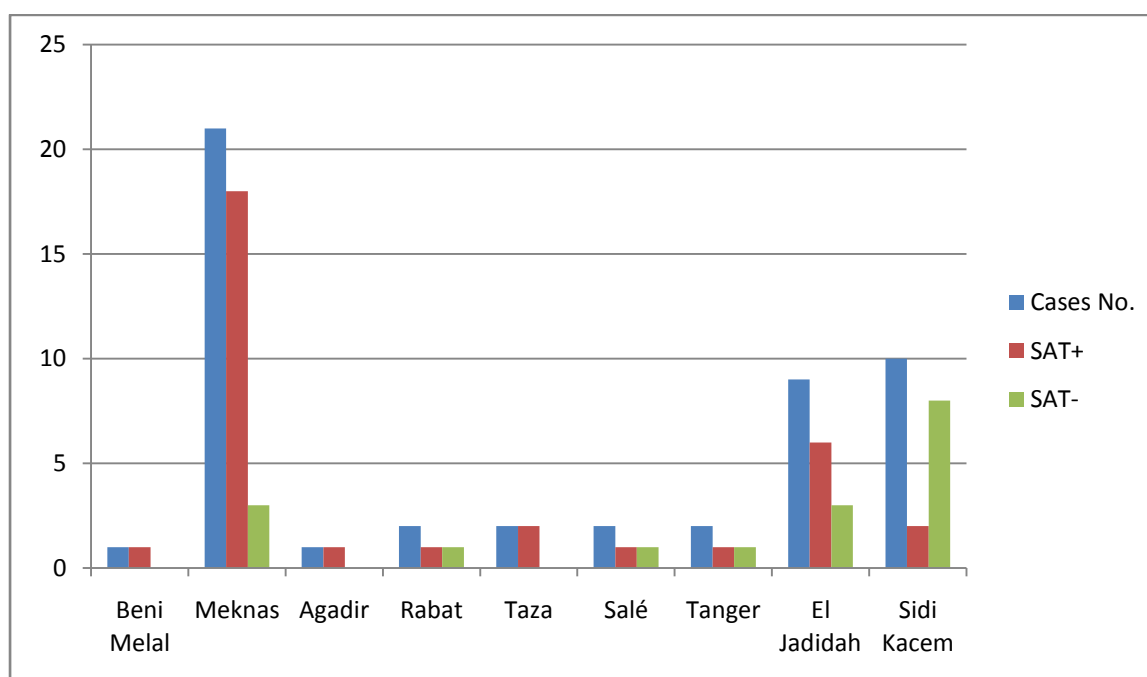
Table 1: Number of cases during 2004-2010, and SAT results.

Year	No. of serum samples	SAT+	SAT-
2004	23	20	3
2005	5	4	1
2006	1	-	1
2007	-	-	-
2008	2	1	1
2009	8	6	2
2010	11	2	9
Total	50	33	17

Table 2: Number of cases in different regions.

Regions	Cases No.	SAT+	SAT-
Beni Melal	1	1	0
Meknas	21	18	3
Agadir	1	1	0
Rabat	2	1	1
Taza	2	2	0
Salé	2	1	1
Tanger	2	1	1
El Jadidah	9	6	3
Sidi Kacem	10	2	8
Total	50	33	17

Figure 1: Number of cases in different regions.



looking for a rapid and simple test, several serological tests have been developed, such as microcapsule agglutination tests, ELISAs using different antigenic preparations or latex agglutination tests. Also molecular diagnosis has been studied for either human or veterinary clinical material(Lilenbaum et al.,2002). Slide Agglutination Test (SAT) is inexpensive, can be performed more quickly and more easily than ELISA, and could be used by the less well equipped laboratories, It should be considered an alternative to MAT or even a replacement for MAT for laboratory screening during the acute phase of leptospirosis(Brandão et al.,1998). Studies in which leptospirosis were diagnosed by IgM Elisa, MAT and SAT, authors demonstrated that the overall results for antibody detection by those three assays were similar. However, SAT and ELISA were statistically more sensitive as initial screening test (Brandão et al.,1998). Chintana Chirathaworn (Chirathaworn et al 2007) compared SAT with LeptoTek Dri-Dot, IgM-ELISA and MAT, for the detection of Leptospira antibodies and demonstrated that SAT and Dri-Dot were more sensitive as initial screening tests than MAT. The follow-up study revealed that SAT is an excellent screening test for the acute phase of leptospirosis. Its sensitivity is high at the beginning of the infection and low in the convalescent phase (Brandão et al.,1998).

In this study, SAT was used to detect leptospira antibodies, from 50 serums during 2004 to 2010. 33 of them (66%) were positive and 17(34%) were negative, 31 (62%) were males and 19(38%) were females. Majority of cases were from Meknas (21 cases). While the Ministry of Health in Morocco indicated that 26 and 77 cases were reported in 2004 and 2005 respectively (Ministry of Health,2005).

In human leptospirosis, adults and males had a greater risk for leptospirosis than children and females(Costa et al.,2015;Katz et al.,2002).Our findings are less than which found in other countries, Thayaparan reported 5267 cases

during 2004-2009 in Malaysia(Thayaparan et al,2013), Katz reported 752 cases in the State of Hawaii (USA) from 1974 through 1998 (Katz et al.,2002).In Taiwan during 2001-2006, 291 cases were confirmed with leptospirosis (Chou et al.,2008) while in China during the period 1991-2010, the total number of confirmed leptospirosis cases was 176,450 (Zhang et al.,2012). In Barbados between 1979 and 1989, 321 cases were diagnosed in 638 patients presenting at a hospital with symptoms of leptospirosis (Cumberland et al.,1999).Despite findings of Leptospirosis in Morocco, no epidemic outbreaks have been recorded (21). However 26 and 77 cases were reported by The Ministry of Health (21).

4. CONCLUSION

Despite Leptospirosis in Morocco is an emerging disease, No epidemic outbreaks have been recorded (Ministry of Health,2005).SAT technique is easy, fast,more sensitive as initial screening testand could be used by the less well equipped laboratories.

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6. REFERENCES

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