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EVALUATION OF SERO-PREVALENCE OF LEPTOSPIRA IN SOUTH GUJARAT REGION, INDIA

Medical Microbiology

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ABSTRACT

Leptospirosis is emerging zoonotic infection in India with highest incidence in Gujarat, Maharashtra, Kerala & Karnataka. In human due to its varied clinical manifestation, diagnosis is difficult & relied mainly on serological test as cultural isolation & molecular diagnosis is tedious & costly. MAT is standard serological test for diagnosis. Present study was conducted on 238 blood samples of Leptospirosis suspected patients in South Gujarat region between years December 2013 to December 2015 in Department of Microbiology at New Civil Hospital, Surat after ethical approval. 16 positive isolates serum samples were subjected to the microscopic agglutination test (MAT). 07 (44%) patients were MAT positive out of 16 patients. Of the 07 MAT positive cases most pre-dominant serovars were australis and autumnalis with highest titre 1:200 and 1:400 respectively observed in 6 (38%) samples followed by icterohaemorrhagiae (highest titre 1:200 in 04 (25%) of the sample). For pomona and hebdomadis the highest titre was 1:100 and 1:50 respectively and was observed in 03 (19%) samples & grippotyphosa and pyrogens showed highest titre of 1:100 and 1:200 respectively and were observed in 02 (12%) samples. Patoc was the most predominant serogroup among non-pathogenic serovar giving the highest titre of 1:400 and was observed in 07 (44%) samples.

KEYWORDS

Leptospirosis, Leptospira, sero-prevalence, Diagnosis, Microscopic agglutination test

INTRODUCTION:

Leptospirosis is an emerging zoonotic infectious disease that is present worldwide. It is caused by pathogenic Leptospira species. Incidence of Leptospirosis is more in tropical regions. In India majority of the cases are reported from the Gujarat, Maharashtra, Kerala, Karnataka, Tamilnadu, Orrisa, West Bengal and the Andaman Nicobar Islands (1). Incidence of leptospirosis increases in Monsoon season. It can infect any mammal & more than one leptospira serovars can cause infection. Rodents are infected most commonly & are these are major natural reservoirs of Leptospira (2-4). In humans it has various clinical manifestations that make diagnosis difficult clinically. Various serological tests are available for the diagnosis of Leptospirosis. Microscopic agglutination test (MAT) is the standard serological test for the diagnosis of Leptospirosis & helpful in identifying predominant strain in that area (5). Most cases reported from India are from the four major states i.e., Kerala, Gujarat, Tamil Nadu, and Maharashtra (6,7) . Prevalence is variable & depends upon region. Present study was carried out to describe the sero-prevalence of Leptospira in South Gujarat region of India.

MATERIAL & METHOD:

Present study was conducted in Department of Microbiology at New Civil Hospital, Surat. Ethical approval was obtained & total 238 blood samples were collected after obtaining consent from suspected patients of Leptospirosis of south Gujarat region (District: Surat, Tapi, Valsad, Navsari) during monsoon season between years December 2013 to December 2015. 10 ml blood was collected from each patient (5ml in heparinised tube for Leptospira culture and 5 ml blood in plain vacuette for serological tests) and processed further as per the protocol. All demographic, epidemiologic and clinical data of enrolled patients were collected. All samples were processed for culture in liquid EMJH media & serological tests were performed. MAT test was performed for the samples as follows:

Sample:

Separated serum sample from whole blood was used.

Panel of antigens:

Batteries of 08 antigens, covering the range of serovars that are expected or likely to be circulating in particular geographical area, where the patient infected, were used. At least one strain of saprophytic serovar (patoc 1) was also included in the panel to act as genus specific antigen to detect infections caused by serovars / strains not yet known to exist in a particular geographical area. Serogroup included in the antigen panel were as follows:Pyrogens (pyrogens), Australis (australis), Autumnalis (autumnalis), Grippotyphosa (grippotyphosa), Semerang (patoc), Pomona (Pomona), Icterohaemorrhagiea (icterohaemorrhagiea) Hebdomadis (hebdomadis), obtained from Regional Medical Research Centre, Indian Council of Medical Research and WHO reference centre for Leptospirosis Port Blair, Andaman.

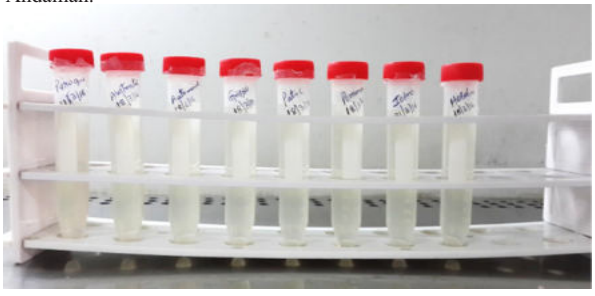

Figure 1: Serovar used for MAT test in South Gujarat region

Preparation of antigens for MAT:

The stock culture collection of Leptospires is best maintained in falcum tubes containing 5 ml of liquid medium. Fresh subcultures are made by inoculating 0.5ml from the each strain / serovar into tubes. At the same time, a loopful of the culture examined by dark field microscopy to confirm the presence of viable leptospires and the absence of contamination and be free from "breeding nests. The inoculated culture are incubated at 30° C and checked for the presence of the growth after 5-7 days. The cultures used as antigens should be checked by MAT against homologous antisera frequently for quality control (8).

Live antigen:

The culture may be used directly as the antigen. The antigens can be used up for one week after 5-7 days of inoculation.

Density of the antigens:

Well grown culture, a minimum density of 2-3 x 10^8 Leptospires per ml should used as antigen. The density can be determined by direct counting using spectrophotometer or by McFarland's scale.

Procedure:

- Fill all 96 wells of microtitre plate with 50 µl PBS.
- Add another 140 µl PBS to the wells of column 2.
- Add 10 µl of serum to the wells of column 2 (dilution is now 1:20) mix and discard100 µl.
- Dilute by pipetting 50 µl from one well to the next, discard the final 50 µl (2->3->4->5->6->7->8->9).
- Add 50 µl Leptospira culture

- Mix thoroughly on a micro shaker or manually with hand
- Incubate for 2-4 hours at 30ºC.
- Read within 2-4 hours.
- Never refrigerate the plate.

READING OF THE TEST RESULTS:

The serum antigen mixtures are examined under a dark field microscope for agglutination. For observation, one drop of a mixture is transferred with platinum loop & pipette from a well to microscopic slide, and examined under dark field microscope with 20x objective without cover slip. The titre is the dilution that gives 50% agglutination, leaving 50% of cells free. Compare with a control suspension of Leptospires diluted 1:2 in PBS without serum at column 1.

Criteria for serological diagnosis of leptospirosis by using MAT:

- Sero-conversion or four fold rise in antibody titre in paired sera.
- A minimum titre of 1:100 or more in a single serum sample

However; the significant titre in the case of single serum samples may vary from one geographical area to other.

RESULT:

238 samples from patients of south Gujarat region with suspected Leptospirosis were included in the study. Average isolation rate from all the samples was 7%. 16 positive isolates serum samples were subjected to the microscopic agglutination test (MAT). The representative Leptospira serovars included in the MAT assay is shown below (Table 1).

Table1: Leptospira Serovar Used For Performing MAT Test

SEROGROUP	SEROVAR
Pyrogenes	Pyrogenes
Australis	Australis
Autumnalis	Autumnalis
Grippotyposa	Grippotyposa
Semaranga	Patoc
Pomona	Pomona
Icterohaemorrhagiae	Icterohaemorrhagiae
Hebdomadis	Hebdomadis

The criteria considered for MAT positivity was either single titres of ≥ 100 in serum sample, or sero-conversion from negative to a titre ≥100, or a four-fold rise in titre for paired samples. 07 (44%) patients were MAT positive out of 16 patients. Of the 07 MAT positive cases most pre-dominant serovars were australis and autumnalis. Amongst the positive 7 MAT sample the highest titre was 1:200 and 1:400 respectively observed in 6 (38%) samples followed by icterohaemorrhagiae the highest titre was 1:200 and were observed in 04 (25%) of the sample. Moreover, for pomona and hebdomadis the highest titre was 1:100 and 1:50 respectively and was observed in 03 (19%) samples whereas grippotyphosa and pyrogens showed highest titre of 1:100 and 1:200 respectively and were observed in 02 (12%) of the total 7 isolates sample tested for MAT (Table 2). Patoc was the most predominant serogroup among non-pathogenic serovar giving the highest titre of 1:400 and was observed in 07 (44%) samples. Dilution used for the MAT testing was 0, 50, 100, 200, 400, and 800 up to 5120.

Table 2 : Serovar Prevalence By MAT Among Leptospirosis Cases

Serovar of Leptospira used for MAT Test	Number of MAT positive for isolates of Leptospira patients serum sample (Out of 16)	Positivity (%)
Patoc	07	44
Australis	06	38
Autumnalis	06	38
Icterohaemorrhagiae	04	25
Pomona	03	19
Hebdomadis	03	19
Grippotyphosa	02	12
Pyrogens	02	12

DISCUSSION:

In this study we have included patients with clinical criteria for Leptospirosis, 13% of cases were seropositive for leptospirosis. Studies from other parts of the country showed a sero-prevalence ranging from 17.8 to 40.5% (9,10,11). In the present study, predominant infecting serovar observed was L. autumanalis (38 %). Tanvi Panwala et al. (12) and Latika shah et al. (13) both the studies have also reported L. autumnalis as predominant strain in south Gujarat. In contrast L. australis was also predominant serovar 38 % observed in present study. L. australis were also reported in both the above study (12,13). Ratnam study has also reported L. autumnalis in Chennai (10) while L. australis was reported as a predominant strain in central and North Kerala by Manju Soman et al. (14). Overall, serological detection of antibodies in patient sera have shown that the most prevalent Leptospira serogroups in South Gujarat have been L. ictrohaemorrhagiae 25%, Pomona and Hebdomadis 19% & Grippotyphosa and Pyrogens 12% (16 of 238 patients). L.icterohemorrhagiae was predominant strain in Mumbai (15), Pune (16) and in Punducherry (17). L. grippotyphosa was found as the predominant serogroup in Andaman Island (18).

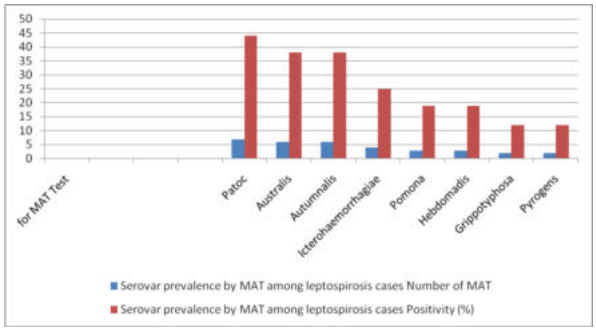

Figure 2: Chart Showing Serovar Prevalence By MAT Among Leptospirosis Cases In South Gujarat

CONCLUSION:

Due to the lack of diagnostic tools, the diagnosis of leptospirosis cannot be easily made in many laboratories. Leptospirosis is often not recognized or is erroneously mistaken for other diseases with similar symptoms (19). The low prevalence (13%) in this study could be due to the fact that we have performed all 3 serological test with only positive isolates of Leptospira as per our inclusion criteria for this study and because of it this is a first study of its kind in the south Gujarat region. Further evaluation by clinicians and better awareness of the disease in conjunction with the availability of a rapid screening test are required for early diagnosis and institution of therapy. Among all 16 isolates serum samples MAT could not be performed in some cases, with the convalescent sample was not available as some patients died or due to other reason after discharge of patients. Moreover, this was a preliminary study with limited sample size, and our findings emphasize the need for a larger study with greater statistical power.

Limitations:

- Strains have to be maintained in culture, which is often very difficult.
- Procedure is complex and time consuming.
- Reading results requires experienced personnel.
- It is not possible to distinguish between IgM antibodies indicative of current infection, and IgG antibodies indicative of past infection.
- Finding of agglutinating antibodies in a single serum sample does not necessarily prove current leptospirosis. An antibody titre may be due to residual antibodies of a past infection. Therefore, the interpretation of a single titre is not easy so a second serum sample is required for demonstrating a raising titre which has a diagnostic significance.
- Calibration of significant titre is essential in the case of single serum specimens.
- False negativity in the early course of the disease.

Abbreviation:

MAT - Microscopic Agglutination Test

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