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Analysis of trend and Seroprevalence of Leptospirae infection by Ig M antibody ELISA in South Gujarat, India: 2017-2021

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ABSTRACT

Introduction

Leptospirosis or Weil's disease is a zoonotic bacterial infection caused by *Leptospira interrogans*. It remains a major public health problem in tropical and subtropical regions. In India, it is endemic, in the southern central, eastern and western region, where heavy monsoon, animal rearing practices, unplanned urbanization and agrarian way of life predispose to this infection. Because of its high rate of incidence, high case fatality ratio, epidemic potential and emergence as an under-recognized severe disease in a highly endemic area, leptospirosis is a crucial public health concern. The diverse clinical presentations of this disease make it essential for the laboratory to play a role in diagnosis. The information on seroprevalence of leptospirosis in western part of India is very limited. This 5-year retrospective observational study provides sero epidemiological demographic details of leptospirosis in south Gujarat.

Aim

To elucidate the status of leptospirosis infection in south Gujarat including seroprevalence, trends, gender and age wise distribution, and seasonal variations from 2017 to 2021. It also intends the diagnostic yield of ELISA and MAT tests for diagnosis of leptospira infection.

Methodology

The present retrospective observational analysis was conducted in the Department of Microbiology, Government Medical College (tertiary care teaching New Civil Hospital), Surat, Gujarat, India. The Leptospirosis section of the microbiology department is the referral center in Gujarat state for leptospirosis diagnostic testing. Samples were tested by Enzyme linked Immunosorbent Assay (ELISA) for Immunoglobulin M (IgM) antibody detection and MAT (Microscopic agglutination test). Demographic and clinical details were obtained and recorded from laboratory request forms using a structured data sheet in using Microsoft Excel software version 2013. The data were entered and analyzed using EpiInfo software version 3.5.1.

Results

Total of 427 blood samples from suspected patients of leptospirosis were received from January 2017 to December 2021. Out of that, 306 samples were tested by ELISA for IgM antibody detection and 143 found presumptive positive for Leptospirosis with prevalence rate of 46.7%. Positivity was maximum 33.6% in 16–30 years of age group, with males outnumbering females in terms of leptospirosis positivity at a ratio of 4.29:1. A seasonal peak of leptospirosis infection was recorded in August every year attributed to heavy rainfall. Majority of the positive cases were from Surat district (53.8%). 272 samples were tested for MAT, 101(37.13%) found to be presumptive positive for leptospirosis.

Conclusion

Leptospirosis is a widespread seasonal disease with varied symptoms confusing with many other diseases which delay the diagnosis. IgM ELISA was found to be the most sensitive, simple and easy to perform test to detect IgM antibodies in acute phase with minimal infrastructure compared to MAT. Periodic presentation of such data on seroprevalence helps to prioritize the health care providers to get sensitized for continuous surveillance and effective control activities.

Keywords: Leptospira; ELISA; Seroprevalence; MAT; Rainfall

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INTRODUCTION

Leptospirosis is one of the most prevalent zoonotic diseases worldwide.[1] Leptospirosis contributes to approximately 48,000 annual deaths with 500,000 cases estimated globally, and remains a major public health problem in tropical and subtropical regions. Tropical regions have favorable environmental conditions for the survival of *Leptospira* spp. in soil and water.[2] In India, it is endemic in southern, central, eastern and western region, where heavy monsoon, animal rearing practices, unplanned urbanization and agrarian way of life predispose to this infection.[3]. The disease is endemic in south Gujarat since 1994. The endemic districts are Valsad, Navsari and Surat. [4]Leptospirosis is caused by bacteria of spirochetes family, the genus *Leptospira* spp. Although many wild and domestic animals can serve as reservoir hosts, the brown rat (Rattus norvegicus) is the common source of human infections. Leptospira entry in humans occurs via small abrasions or other breaches of the surface integument. [5] Human infection may occur through either direct contact with an infected animal or through indirect contact via soil or water contaminated with urine of an infected animal. Occupations like veterinarians, abattoir workers, farm workers, animal handlers, sewer workers, scientists and technologists handling animals in laboratories or during fieldwork are at increased risk of having infection. [6] Incubation period of leptospira ranges from 3 to 30 days; usually 10- 12 days. [7] The clinical features of leptospirosis are highly variable and are divided into four categories: 1. A mild, infectious-like illness 2. Weil's syndrome, characterized by jaundice, renal failure, hemorrhage, and myocarditis 3. Meningitis/meningoencephalitis 4. Pulmonary hemorrhage with respiratory failure. [8]Leptospira may be detected in blood, urine or tissues by culture, dark field microscopy, immuno-staining, PCR or by serological tests like microscopic agglutination test (MAT) and enzyme-linked immunosorbent assays (ELISA) by detection of specific antibodies. The diagnosis of leptospirosis is not usually based on bacterial detection as it is difficult to isolate and maintain, but is mainly based on serology. [9] Serological techniques such as microscopic agglutination test (MAT) and ELISA are the most commonly used means for confirming a diagnosis of leptospirosis. ELISA remains the most preferred cost-effective serological method with both sensitivity and specificity of 95%. [10] This present study is done to elucidate a comprehensive overview of the seroprevalence of leptospira infection in South Gujarat districts where agriculture-related activities form a major occupation of the population and are at risk of acquiring leptospirosis. It intends to find out the diagnostic yield of ELISA and MAT tests for leptospira infection. It also contributes the trend analysis, gender and age wise distribution and seasonal variations of leptospira infection in South Gujarat and other parts of Gujarat during the year 2017 to 2021.

Materials and Methods

This retrospective observational study was conducted at the microbiology department, tertiary care teaching hospital, Surat, Gujarat. The Leptospirosis section of the microbiology department is the referral center in Gujarat state for leptospirosis testing. We cater diagnostic services like staining, dark ground microscopy, culture, PCR, MAT (Microscopic agglutination test), IgM Antibody ELISA, Rapid test for leptospirosis infection as well as maintaining records of positive cases and their isolates for future use. Leptospira infection is more commonly seen in South Gujarat mainly Surat, Valsad, Navsari, Tapi, Narmada districts. We receive majorly samples from these districts and also from other parts of Gujarat state. Total of 427 blood samples from suspected patients of leptospirosis were received in the microbiology department from January 2017 to December 2021.The study protocol was approved by the institutional ethical committee (GMCS/STU/ETHICS-2/Approval/9692/23). Informed written consent was waived because the study was a retrospective data analysis.

Inclusion Criteria

Blood samples (5ml) were collected in plain vacuette from the patients of different age groups who were suspected clinically of leptospirosis and presented with acute febrile illness with headache, myalgia and prostration associated with any of the following features i.e. Conjunctival suffusion ,Meningeal irritation ,Anuria or oliguria and/or proteinuria ,Jaundice , hepatosplenomegaly, hemorrhagic manifestations ,Skin rash , Acute respiratory distress syndrome and a history of exposure to infected animals or an environment contaminated with animal

urine, occupational history. Single /paired sera of acute illness of leptospirosis and after 10 days 2nd sample as convalescent sera requisites for ELISA Ig M antibody testing for leptospirosis are included in study.

Exclusion criteria

Patients with other acute or chronic illnesses such as malaria, typhoid, dengue, hepatitis or with other comorbid diseases were excluded from the study.

Study Procedure

Blood samples along with duly filled laboratory request form were received in the microbiology department. Blood samples were allowed to clot at room temperature and centrifuged at 2000 RPM for 10 minutes. Separated serum sample was used for leptospirosis testing. Clinically suspected leptospirosis patient's serum samples were tested for Ig M antibody detection by ELISA using commercial kits (Panbio Leptospira IgM Elisa, Abott Diagnostics, Korea) as per manufacturer's instructions and results were recorded. Samples were also tested by MAT (Microscopic agglutination test), PCR, and Rapid test as per requisition.

MAT (Microscopic agglutination test) Procedure: Doubling dilutions of serum from 1 in 25 to 1 in 6400 were prepared by adding PBS (Phosphate buffered solution) in 96 well flat-bottomed microtiter plates. Fill all 96 wells of microtiter plate with 25µl PBS. Add another 23 µl PBS and 2 µl of serum to the wells of column 2 (now dilution becomes 1:25). mix and transfer 25 µl from one well to the next, discard the final 25 µl. Add 25 µl leptospira cultures to all wells. One of the wells included only the antigen without addition of antibody and served as the antigen control. The final dilutions after adding the antigen were from 1 in 50 to 1 in 12800. Mix thoroughly on a micro shaker. Incubate at 37°C for 2 hours.

Reading of the test results: The serum antigen mixtures are examined under a dark field microscope for agglutination. For observation, one drop mixture is transferred with a pipette from a well to a microscopic slide and examined under a dark field microscope with a 20x objective without cover slip. The reported titre was calculated as the reciprocal of the highest dilution that agglutinated at least 50% of the cells for each serovar or a reduction in the number of leptospiral cells as compared to the antigen control was taken as end point titer. A titer of 1 in 100 or more was considered significant. [11] Serovars tested were L. autumonalis, L. patoc, L. australis, L. pyrogenes, L. Icterohemorrhagiae, L. Pomona, L. grippotyphosa, L. hebdomadis.

Data collection and analysis

Demographic and clinical details were obtained and recorded from laboratory request forms. A structured data sheet was created using Microsoft Excel software version 2013. The data were entered and analyzed using EpiInfo software version 3.5.1. The analysis was presented as frequency and percentage distributions. Tables and Graphs are used to describe the overall as well as specific trends of leptospirosis prevalence during the study period. Data were analyzed retrospectively to know trend of seropositivity of leptospirosis infection, age and gender wise dissemination, seasonal variations and epidemiological distribution of leptospirosis infection by using ELISA Ig M antibody positive result as an indicator of infection. [1,11]. Confidentiality is maintained throughout by avoidance of use of personal details such as name and other personal identifiers during the formulation of the report. The study protocol was approved by the institutional ethical committee (GMCS/STU/ETHICS-2/Approval/9692/23).

Results

Total of 427 blood samples from suspected patients of leptospirosis were received in the microbiology department from January 2017 to December 2021. Out of that, 306 samples were tested by ELISA for IgM antibody detection for leptospirosis in acute phase. We found 143 of 306 to be positive by IgM ELISA as presumptive positive for leptospirosis with prevalence rate of 46.7%. Out of 306, we received 80 convalescents' sera after 10-14 days of primary sample from presumptive positive patients for IgM ELISA testing to confirm the diagnosis of leptospirosis. Out of which, 70 (87.5%) were confirmed cases of Leptospirosis.

Table-1: Year wise data of suspected leptospirosis cases and ELISA positive cases

Year	Total No. of suspected Leptospirosis (n=427)	Total No. of samples tested by ELISA (n=306) Acute Sera	Total No. of samples Positive by ELISA (n=143) Positive	Total No. of samples tested by ELISA (n=80) Convalescent Sera	Total No. of samples Positive by ELISA (n=70) Positive
2017	134 (31.4%)	100	56	32	28
2018	95 (22.2%)	70	29	17	14
2019	108 (25.3%)	70	31	19	17
2020	35 (8.2%)	28	13	5	4
2021	55 (12.9%)	38	14	7	7

During the study period, highest suspected cases 134(31.4%) and seropositive cases 56 (41.7%) was noted in 2017. [Table1]. Suspected dengue cases had declined in 2020 (8.2%) due to Covid Pandemic as there was a lockdown period and less activity/mobilization.Males were affected more commonly than females by a ratio of 4.29:1. Seropositivity was 116 (81.1%) among males which was higher compared to females which was 27(18.9%). Maximum positivity of 33.6% was in the age group of 16–30 years, followed by 27.9% in 31-45 years of age group males. [Table 2]

Table-2: Age wise gender distribution of leptospirosis positive cases

Age Groups (Years)	No. of the ELISA positive cases in males n= 143(%)	No. of the ELISA positive cases in females n= 143(%)	Total No. of ELISA positive cases n= 143(%)
1 – 15	1 (0.7%)	2 (1.4%)	3 (2.1%)
16 – 30	48 (33.6%)	7 (4.9%)	55 (38.5%)
31 – 45	40 (27.9%)	11 (7.7%)	51 (35.6%)
46 – 60	20 (14%)	6 (4.2%)	26 (18.2%)
> 60	7 (4.9%)	1 (0.7%)	8 (5.6%)

Majority of samples for ELISA testing were received from Surat district. Out of total 306 samples, 154 (50.3%) samples were from Surat district and 77 (53.8%) samples turned positive during 2017-2021. A total of 100 (32.7%) samples were received from other districts of south Gujarat (Navsari, Valsad, Tapi, Bharuch, Narmada), out of which 47 (32.9%) samples were positive by IgM Ab ELISA for leptospirosis. We also received 52 (17%) samples from other districts like Ahmedabad, Vadodara, Nandurbar, miscellaneous districts (Silvassa,Mahuva,Daman, Dadra Nagar Haveli,Chota Udepur, Bhavnagar, Rajkot, Patan) out of which 19 (13.3%) were positive. [Fig 1].

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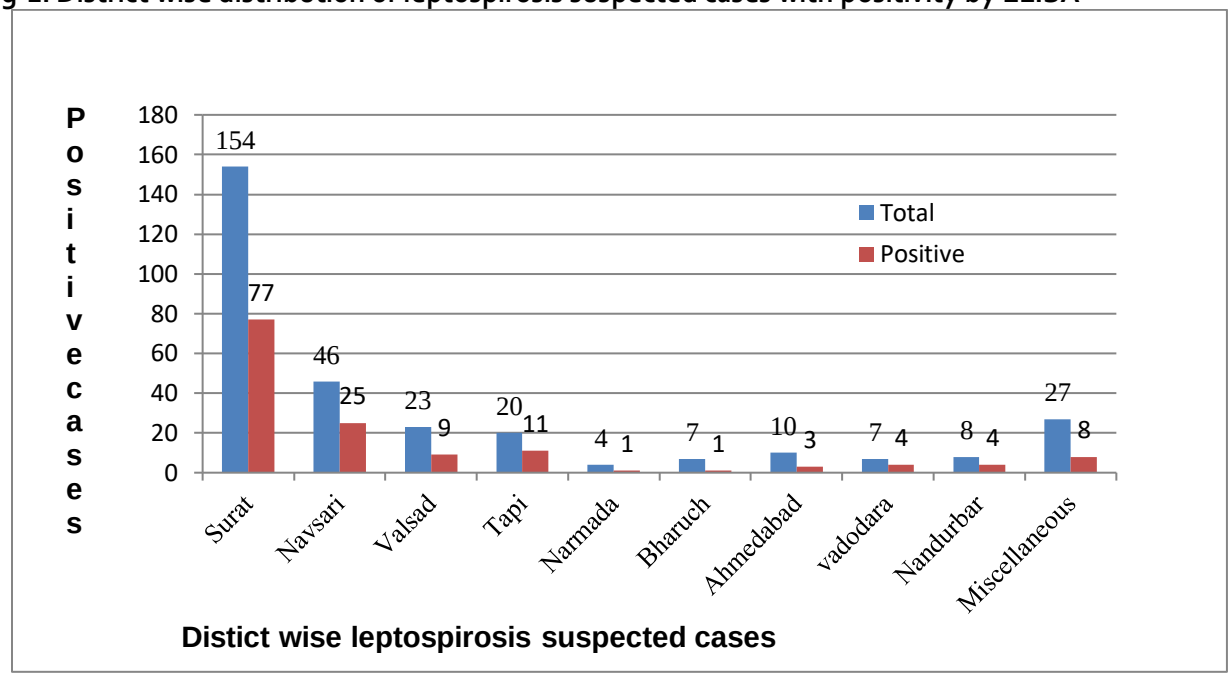
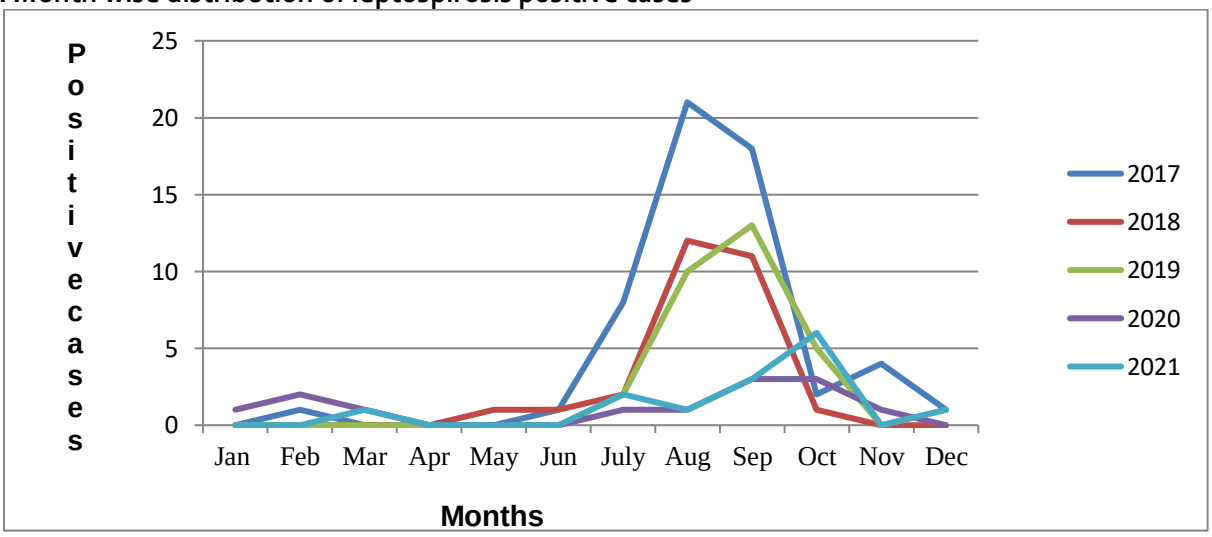
Fig-1: District wise distribution of leptospirosis suspected cases with positivity by ELISA



As the monsoon in south Gujarat starts from June and last till October, leptospirosis positivity starts rising from the end of June, reaches to the peak in August, September and starts declining from the end of October every year. A seasonal peak of leptospirosis infection was recorded maximum in August every year. [Fig 2]

Fig-2: Month wise distribution of leptospirosis positive cases



MAT (Microscopic agglutination test) test was also performed in most of the leptospirosis suspected cases requisite for ELISA Ig M antibody testing. Considering MAT titres of ≥1:100 as positive , out of 272 samples requisite for MAT ,101(37.13%) found to be presumptive positive for leptospirosis. [Table 3] Out of 101 MAT positive samples, 88 (87.1%) were positive by IgM ELISA, 7 (7%) were negative by ELISA and 6 (5.9%) samples were not requested for ELISA testing. 40 samples were negative by MAT but Positive by ELISA. We also received 79 Convalescents sera for MAT testing to see fourfold rising titre, 69 (87.3%) turned Positive as confirmed case of leptospirosis. Serovars detected were- L. autumonalis and L. australis as a predominant strain followed by L. pyrogenes and L. Icterohemorrhagiae.

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The other serovars observed were L. pomona, L. grippotyphosa, L. hebdomadis.

Table-3: Year wise data of suspected leptospirosis cases and MAT positive cases

Year	Total No. of suspected Leptospirosis (n=427)	Total No. of samples tested by MAT (n=272) Acute Sera	Total No. of samples Positive by MAT (n=101) Positive	Total No. of samples tested by MAT (n=79) Convalescent Sera	Total No. of samples Positive by MAT (n=69) Positive
2017	134 (31.4%)	97	41	31	27
2018	95 (22.2%)	58	22	17	14
2019	108 (25.3%)	66	23	19	17
2020	35 (8.2%)	18	05	05	04
2021	55 (12.9%)	33	10	07	07

Table-4: Diagnostic yield of ELISA and MAT testing in acute and convalescent phase

	ELISA	MAT
Acute Sera	143/306 (46.7%)	101/272 (37.1%)
Convalescent Sera	70/80 (87.5%)	69/79 (87.3%)

Discussion

Leptospirosis is a zoonotic bacterial infection with humans as the accidental host. Almost all mammalian species can shelter leptospires in their kidneys and act as a source of infection to human beings and other animals. Rodents were the first recognized carriers of leptospires who sheds leptospires throughout their lifespan without clinical manifestations. [12] The core determinants associated with leptospirosis are temperature, humidity, rainfall, soil structure, presence of carrier animals prevalent in the region. [13, 14] Leptospirosis is endemic in South Gujarat due to various predisposing factors such as heavy rainfall, humidity, clayey soil, paddy and sugarcane fields which enhances water logging, and rodents which are one of the major biological constraints in rice paddies. So, farmers who work in irrigated fields acquire the infection through contaminated urine of rodents & cattle. [4, 15, 16] Leptospirosis typically has two clinical forms: anicteric and icterohemorrhagic. Fever, chills, headache, severe myalgia, conjunctival suffusion, anorexia, nausea, vomiting, and malaise usually characterizes acute leptospirosis. Pulmonary involvement and acute kidney injury are the main causes of death in leptospirosis. [17] Because of the wide diversity of clinical signs, diagnosis of leptospirosis is difficult and depends upon a variety of microbiological laboratory tests.

Out of 427 total blood samples from suspected patients of leptospirosis, 306 were tested for IgM antibody detection by ELISA and 143(46.7%) were presumptive positive for Leptospirosis. Out 143 presumptive positive samples, 80 (56%) convalescent sera were received in which 70 (87.5%) were confirmed leptospirosis patients with high titre.

Overall seroprevalence of leptospirosis by Ig M ELISA in acute sera was 46.7% during study period. A similar study on IgM ELISA done by Sandhya et al [2] reported 23.5%, 22% by Shukla et al [18] and 12.7% by Deshmukh et al [12]. Variation in seropositivity depends on endemic nature of organism and geography as well as rainfall. Seropositivity by Ig M ELISA was 74% in 2012 [11], 56% in 2017, 41.4% in 2018, 44.3% in 2019, 46.4% in 2020 and 36.8% in 2021. This declining trend of seroprevalence rate of leptospirosis indicates the impact of preventive strategies implemented by municipalities in reduction of leptospirosis cases over the years. This may be attributed to several factors such as increase in community awareness in endemic zones, improved hygienic practices, use of safe drinking water, rodent control measures and chemoprophylaxis in the early phase of monsoon.

Seropositivity among different age groups was similar throughout the study exposure. Age group 16-30 years was most commonly affected with seropositivity of 38.5% followed by 35.6% in 31-45 years. This data is consistent with studies done by Shukla et al [18] and holla et al [19] where 20-60 years of age group are most commonly affected with prevalence rate of 64% and 40% respectively. Positivity Rate was 18.2 % in 46-60 years and 5.6% in >60 years of age groups.

Positivity among male was 81.1% which was high as compared to females which was 18.9%. Male patients showed more prevalence of this disease and male: female ratio was 4.29:1 which is comparable with the study by Agrawal et al [10] who reported 4.08:1. This can be due to the fact that male of reproductive age groups is the working population who acquire the infection mostly during field work, animal handling and outdoor activities compared to females. [19]

In the present study, maximum cases of leptospirosis was observed during monsoon season starting from end of June till October, which could be attributed to its correlation with rainfall, flooding , clogging ,humidity, temperature variations, and agricultural activities.[3,13,14,16] As leptospira are shed in animal urine, survive in water or soil , increased moisture and humidity during monsoon, and capability to form biofilm helps organism to stay for long periods in aquatic ecosystems and disease transmission.[14,20] This helps to prioritize the health care providers to get sensitized for continuous surveillance and effective control activities. These includes health education, use rubber shoes and gloves to prevent contact with contaminated water, prevent draining of urine from cattle sheds directly into water bodies, rodent control, mapping of water bodies for establishing a proper drainage system and chemoprophylaxis in high-risk population from where clustering of cases has been reported. [12,21] Almost 53.8% of leptospirosis seropositive cases were from Surat district compared to other districts of South Gujarat (33%). Miscellaneous districts showed prevalence rate of 13.2%. The agro-climatic conditions for south Gujarat favor endemicity for leptospirosis attributed to heavy rainfall, clay soil and high-water bodies. [4, 15, 22] The present study revealed that IgM ELISA positivity to detect leptospira infection in acute phase was 46.7% compared to MAT which was 37.13%. [Table/Fig-6] This finding is similar with the studies done by Prakash K et al [23] and Niloofa R et al [24]. Seropositivity remains almost the same in convalescent sera by ELISA and MAT. So, IgM ELISA was found to be the most sensitive, simple and easy to perform test to detect IgM antibodies in acute phase with minimal infrastructure. MAT is gold standard test for leptospirosis but has some limitations with less sensitivity in the early phase of the disease, as it detects both acute and chronic immunoglobulins .It is labour intensive and complicated procedure with continual maintenance of Leptospira strain for preparing live antigen.[2,3,18]Most common serovar of leptospira detected was L.autumonalis and L. australis followed by L.pyrogenes and L.Icterohemorrhagiae which is consistent with previous study done by Panwala et al in 2012[11].

Conclusion

Leptospirosis is a widespread seasonal disease associated with rainfall, humidity, farms, animal handling, and rodents with preponderance in males of reproductive age group. As the disease symptoms vary from mild flu like illness to a wide variety of clinical syndromes confusing with many other diseases, early detection of the infection during the acute phase by Ig M ELISA helps to establish the diagnosis and hence reduction in the mortality associated with it. Furthermore, training and education of primary care physicians and health care

workers, supply of serological test kits like rapid test, ELISA test till peripheral level, timely reporting and publication of such data of trends are required in understanding of this problem and its prevention.

Limitations

We were not able to collect the paired (convalescent) sera from some of the presumptive positive patients to know the true scenario of infection and diagnostic yield of serological tests.

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