ORIGINAL ARTICLE

OUTBREAK SURVEILLANCE REPORT ON PULMONARY LEPTOSPIROSIS AFTER A HEAVY FLOODS DURING 2006 IN SOUTH GUJARAT

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ABSTRACT

Background: During the heavy rainfall season in the Surat district of South Gujarat India, from July to October 2006 an outbreak of leptospirosis occurred.
Aim: This article reports the exposure of leptospirosis in this post flood outbreak. In total 1,258 patients of New Civil Hospital in Surat were included, based on their clinical signs and symptoms for leptospirosis. Severe pulmonary hemorrhages were observed in the imperative form in most cases encountered during this season.
Method: Laboratory investigation was carried out using rapid diagnostic tests like Leptocheck WB, Serion IgM ELISA and real-time PCR and they were evaluated for the outbreak investigation in comparison with the microscopic agglutination test (MAT)
Observation and Results: The predominant serovars encountered by the gold standard MAT were *autumnalis*(46%), *australis*(38%), *pyrogenes*(30%), *cynopteri*(20%), *icterohemorrhage*(8%) and g*rippotyphosa*(1.6%). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of rapid tests were analyzed, Leptocheck WB (91%, 78.4%, 83% & 88.3%), Serion IgM ELISA (92.2%, 89.4%, 90.3% & 91.6%) and Real time PCR (90.3%, 91.6%, 96.02% & 96.02%) using statistica (6.0). The incidence of the disease was greater during the month of August (41.41%) and September (52.94%) with a relative risk of 33.5 in Surat.
Conclusion: This implicates the impact of the heavy rainfall and flood as the cause for severe outbreak of leptospirosis among the urban population of Surat district. Frequently contaminated environmental exposures due to urbanization and industrialization were speculated as major cause for this severe epidemic during heavy floods, which entails preventive strategies and prompt treatment against leptospirosis under such outbreak circumstances.

Keywords: Leptospirosis, outbreak, MAT, Real time PCR, Leptocheck, IgMELISA

INTRODUCTION

Leptospirosis is a zoonotic disease having worldwide distribution and is caused by Genus *Leptospira*. The causative agent *Leptospira* is mainly transmited to humans through the environment or direct contact with urine from infected animals[1]. Infections with pathogenic *Leptospira* are increasingly recognized as a common cause of acute febrile illness in tropical environments[2]. The incidence of pulmonary involvement in Leptospirosis has been reported to be increasing and among 70% of the patients, alveolar hemorrhages dyspnea and hemoptysis are the predominant manifestations[3]. It is most common in tropical countries like Nicaragua [4, 5], India[6] and Thailand[7]. Pulmonary involvement in leptospirosis was first observed in India during outbreaks in Andaman Islands[8]. In Australia also pulmonary hemorrhage has been reported in patients with leptospirosis[9]. In past two decades, there is an increase in the number of cases of leptospiral pulmonary hemorrhages especially from Southeast Asia. This is mainly due to longer survival of *Leptospira* in environments with warm and humid conditions. Leptospirosis is a seasonal disease and the incidences mainly occur during the rainy season. The usual portal of entry is through abrasions or via the conjunctiva or intact skin after prolonged immersion in water [10,11]. Water-borne transmission has been documented in outbreak situations of Leptospirosis, usually after flooding. Apart from seasonal epidemics, the flood related outbreaks have increased the attentiveness of the epidemiologists to identify the cause and source of Leptospirosis.[12, 13]

Leptospirosis is a disease with protean manifestations, ranging from subclinical cases in the anicteric form to

the severe icteric form known as Weil's disease are characterized by a fulminant course with rapid onset of hepatic and renal failure and high mortality. Incubation period varies from 7 to 12 days but may range from 2 to 20 days. Leptospirosis classically presents as a biphasic illness. The first phase of the disease is commonly referred to as the septicemic phase. It is characterized by fever, headache, myalgia, conjuctival congestion and a host of non-specific features that may include mild cough, lymphadenopathy, rash, anorexia, nausea, and vomiting. This phase is followed by a brief febrile period of variable duration that, in turn, is followed by the immune phase of the illness.[2, 9] The common organs involved during this phase are the liver, lungs and kidneys. Both organ derangements are reversible.[14,15]

Leptospirosis diagnosis mainly rely on serological methods, Microscopic Agglutination Test (MAT) which remains useful for epidemiologic studies, identification of strains, assessment of the probable infecting serovar and confirmation of illness for public health surveillance. [16] In this report we discussed our experience of 2006 post flood Leptospirosis outbreak in Surat and the clinical presentation of the cases. The rapid diagnostic tests like Leptocheck WB, Serian IgM ELISA and real time PCR were evaluated in comparison with Microscopic Agglutination Test (MAT) during this severe disaster condition.

MATERIALS AND METHODS

Surveillance site

The City of Surat is located in the Southern part of Gujarat at 21° 15' N latitude and 72° 52' E longitude on the Southern bank of Tapti River, where the total population of Surat is approximately 4 million. During summer the temperatures range from 37.78°C to 44.44°C. The climate is pleasant during the monsoon season, while autumn is temperate. The winters are not very cold but the temperatures in January range from 10°C to 15.5°C. The average annual rainfall of the city has been 1143 mm. During August 2006 there was heavy rainfall all over India, but it was heavier in Madhya Pradesh state. The sudden release of a huge amount of water from the Ukai dam led to over 80 per cent of Surat going under water. More than 2 million people were trapped in their houses without food and drinking water for four days and four nights. The floods that ravaged Surat on 7th August left millions of people homeless and marooned thousands of animals. The rains disrupted communications, power and water supplies to the city. The transport system between Surat and other districts were cut off because of the raging waters from the Tapti river. As water receded in Surat the entire city was transformed into a garbage dump, with two feet of mud and muck on the streets. Hundreds of Leptospirosis cases were reported during the subsequent weeks which accounted for the large epidemic.

Patients and criteria used for clinical diagnosis

All the 1258 patients admitted, with clinical suspicion for Leptospirosis was included in the investigation. Among them 744 were males and 614 were of females. Investigations were carried out during the outbreak and observed that all patients had a high grade fever, headache and generalized body aches, associated with at least any one of the following sets of signs and symptoms. They included, according to criteria laid down by Indian Leptospirosis Society, a) jaundice, b) oliguria, c) cough, hemoptysis and breathlessness, d) neck stiffness with altered sensorium, and e) hemorrhagic tendencies including conjuctival suffusion and others.

Case confirmation by serological examination

As a part of the surveillance protocol, acute and convalescent- phase serum samples were obtained from suspected patients within 24 hours of admission. Among the cases, 675 paired sera were possible and they were collected in a mean interval of (> 14 days). Patients fulfilling any of the following criteria were considered as cases of leptospirosis: i) positive isolation of leptospires from blood or urine, ii) seroconversion or four fold titer in MAT for those with paired samples, iii) A titer of 1:80 or more with a positive IgM ELISA (titer of 1:80).

Serovar Specific microscopic agglutination test (MAT)

MAT was performed on the samples using eleven live leptospiral strains as antigens. The strains belonged to the serovars *australis* (JezBratislava), *autumnalis* (Bankinang) *ballum* (Mus127), *sejroe* (Hardjoprajitno), *grippotyphosa* (MoskvaV), *canicola* (HondUtrechIV), *hebdomadis* (Hebdomadis), *pomona* (Pomona), *patoc* (PatocI), *pyrogenes* (Perepelician), *icterohaemorrhagiae* (RGA). All the strains were obtained from Leptospira WHO Reference Centre, Port Blair and maintained with periodical subculture in Ellinghausen McCullough Johnson and Harris (EMJH) medium (Difco) at Department of Microbiolgoy, Government Medical College, Surat. The seven days old cultures having a concentration of 1-2x10^8 were used as antigen as per standard procedures.[17]

Rapid genus specific tests

Rapid genus specific tests like Leptocheck-WB (Zephyr Biomedicals, India) and Serion IgM EISA (Serion GmbH, Germany) were performed as per the manufactures instructions.

Real Time PCR assay

Total DNA from human serum (200 µl) was prepared using QIAamp DNA Mini Kits (QIAGEN, USA) according to the manufacturer's instructions. The primers and probes were designed from alignments of available *Leptospira spp. LipL41* sequences obtained from the GenBank nucleotide sequence database. The program used was Primer Express™ (Applied Biosystems, USA). For real time PCR, 5 µl of DNA

was added to the 45 µl TaqMan Universal PCR Mastermix Mix (Applied Biosystems, USA) in a final concentrations of 3 pmol/µl of each primer and 2 pmol/µl of the FAM-TAMRA labelled probe. A negative control without added template in the above reaction mixture, was used as a control to detect the presence of contaminating DNA. Amplification and fluorescence detection was conducted in an ABI Prism 7700 sequence detector (Applied Biosystems, USA) with a program of 40 cycles, each cycle consisting of 95°C for 15 seconds and 60°C for one minute as per the manufacturer's instructions.

RESULTS

This study has been conducted to investigate the post flood prevalence of human Leptospirosis in and around Surat. Of the 1,258 suspected cases from Surat, Navsari and Valsad highest incidence 1103 (87.6%) was observed from Surat. In total cases about 801 patients were confirmed with Leptocheck (63.6%), 690 by IgM ELISA (54.8%), 702 by Real Time PCR (55.8%) and 675 MAT (53.6%). The 121 patient's deaths that were reported caused a mortality of 9.61%.

Table 1: Frequency of clinical signs among the suspected cases of leptospirosis from Surat, Navsari and Valsad

Clinical signs	Surat 1103 (87.6%)	Navsari 110 (8.74%)	Valsad 45 (3.57%)	Total *n* = 1258
Fever	1010 (92)	98 (89)	36 (80)	1144 (91)
Mylagia	980 (89)	98 (89)	34 (76)	1112 (88)
Headache	988 (79)	95 (86)	32 (71)	1125 (89)
Jaundice	450 (41)	32 (29)	18 (40)	500 (40)
Nausea/Vomiting	972 (88)	65 (59)	29 (64)	1066 (85)
Meningeal signs	210 (19)	30 (27)	12 (27)	252 (20)
Conjunctival suffusion	740 (67)	28 (25)	8 (18)	776 (62)
Pneumonial/ respiratory	326 (30)	14 (13)	8 (18)	348 (28)
Hemorrhage	678 (61)	28 (25)	16 (36)	722 (57)
Hemoptysis	320 (29)	11 (10)	9 (20)	340 (27)

The most frequent symptom encountered was fever in all the three places; nearly 91% of total cases had fever. Apart from this myalgia, nausea and vomiting, headache and conjunctival suffusion were other common symptoms observed among the patients. Icteric type of illness was associated with 40% of the patients and 57% of patients were reported with severe pulmonary hemorrhages (Table.1).

Table 2: Age and sex wise distribution among the leptospirosis cases during outbreak investigation

Age	Male	Female	Total	%
0-9	17	11	28	2.22
10-19	164	74	238	18.91
20-29	214	144	358	28.45
30-39	189	192	94	30.28
40-49	64	30	94	7.47
50-59	51	41	92	7.31
60-69	44	12	56	4.45
70-79	9	7	16	1.27
80-89	11	3	14	1.11

Age and sex distribution of the patients were analyzed and it revealed most of the patients were in the age group of 10-59 and predominantly males (Table.2). Seven hundred and forty four (59%) were males and five hundred and fourteen were (41%) were females. In this current outbreak situation, the relative risk was estimated to be higher in Surat (33.50), followed by Navsari (19.3) considering the Valsad with minimum number of observed cases as a reference group (Table.3). Seasonal distribution of the cases observed exhibited September (666) as a predominant month followed by August (521), July (50) and October (21) (Table 4). Incidence of leptospirosis observed was higher during heavy rainfall (July-October) in Surat compare to Navsari and Valsad. Crystalline Penicillin 20 lac IU I/V 6 hourly / Rantac I/V 12 hourly was practiced for the treatment of the suspected cases for leptospirosis and it has responded well.

Table 3: Relative risk among the leptospirosis cases of Surat, Valsari and Navsad

Area	No. of cases (%)	Relative risk	Death reported	% Mortality
Surat	1103 (87.6)	33.50	85	6.75
Navsari	110 (8.74)	19.30	29	2.30
Valsad	45 (3.57)	1.0	7	0.55
Total	**1258 (100)**		**121**	**9.61**

Table 4: Month wise distribution of leptospirosis cases during the outbreak investigation

Months	July	August	September	October
Surat	22	453	614	14
Navsari	21	54	31	04
Valsad	07	14	21	03
Total	**50**	**521**	**666**	**21**

The predominant serovars encountered for the outbreak was determined by MAT. Serovars like *autumnalis* (46%), *australis* (38%) and *pyrogenes* (30%) were observed as the predominant circulating serovars with a highest titre of 1:1280 (Table.5). Rapid tests like Leptocheck, Serian IgM ELISA and real time PCR were evaluted in an outbreak situation for leptospirosis (Table 6).

The performances of the rapid test were evaluated based on their sensitivity and specificity of each test in comparison with the gold standard Microscopic Agglutination Test. For Leptocheck WB sensitivity and specificity observed was 91% and 78.4% with a positive and negative predictive value of 83% and 88.3%. For IgM ELISA it was observed as 92.2% sensitivity and 89.4% specificity along with positive and negative predictive value of 90.3% and 91.6%. Among all the three tests the performance of real time PCR was admirable with a sensitivity of 96.5% and specificity of 95.5% and its positive and negative predictive value were determined as 96% and 96%.

Table 5: Distribution of predominant leptospiral serovars among the leptospirosis cases during outbreak investigation

Serovar	Number	%
Autumnalis	357	46
Australis	298	38
Pyrogenes	238	30
Icterohaemorrhagiae	66	8
Cynopteri	158	20
Grippotyphosa	13	1.6
Patoc	13	1.6

Table 6: Evaluation of various diagnostic methods among the Leptospirosis cases during outbreak situation

Tests	Positive cases (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Rapid Leptocheck WB	801 (63.6)	91	78.4	83	88.3
SERION IgM ELISA	690 (54.8)	92.2	89.4	90.3	91.6
Real Time PCR	702 (55.8)	96.5	95.5	96	96

DISCUSSION

The diagnosis of acute undifferentiated febrile illness is difficult in tropical settings where many possible agents can be responsible for infectious disease outbreaks. Such was the case with the outbreak of leptospirosis in Andaman Islands and Nicaragua during the year 1995 [4,5], when thousands of patients developed acute undifferentiated febrile illness and several dozen died of severe pulmonary hemorrhages as the predominant signs and symptoms[8]. Surat is a densely populated area with urbanization combined with industrial developments and prone to garbage and urban wastes that posed a severe impact after this heavy flood. As water receded the entire city was stinking with mud heaps and soon rotten household perishables were also dumped on the streets. The contact between the infectious agent and susceptible individuals can occur distant from the supported foci or the case residence because of rodent and human circulation especially during floods. During the dry periods, high leptospira concentrations in the soil are limited to few meters around the waste accumulation sources. But during the heavy flood conditions it increased the possibilities for the infectious agent to spread and reach a distant area caused by the movement of water. At the same instance, this same flood dilutes both the agent and also its infectivity at a great distance from the sources. This may be evident from our results for the reason by which the Surat city has shown higher relative risk to leptospirosis when compared to other regions like Navsari and Valsad. The scattering of flood water upholds the agent's contact with the population group, so that the individuals with no previous contact with the leptospira and fall under low risk group to leptospirosis may also subjected to infection due to this flood. However, a high prevalence of infection was detected among the individuals living in close proximity and with frequent contact with the agents. Thus, a shift in seropositivity can be predicted in such flood situation over the normal periods. Similar reports were noticed in Reo de Jeneiros, Western region in 1996, where high incidence rates were identified in areas that had precarious sanitation conditions and were vulnerable to floods [18,19]. According to the report, densely populated urban areas displayed an excess of leptospirosis cases around waste accumulation sites. It was observed that in Surat, the incidence was greater during the months of August and September particularly may be because of the deficiency of convenience to the people to reach health care personnel or a hospital under the severe rain fed circumstances and flood havoc. Rather sources of infection may be due to the overflowing of water bodies like ponds, pools, domestic sewage which is often susceptible to urine contamination by the carriers of leptospires like rodents, swine, dogs and cattle.

During this outbreak in and around Surat district of South Gujarat, most of the cases admitted were having high grade fever, headache and generalized body aches, associated with pulmonary hemorrhagic conditions and conjuctival suffusion. Large numbers of cases were observed in Surat city followed the flood with nearly 675 confirmed cases along with 121 deaths. The case fatality rate reported was significant in South Gujarat during the last 13 years of epidemic history. Particularly in patients confirmed with leptospirosis, they were

mostly developed with severe pulmonary haemorrhages in comparison to the previous years. The correlation between clinical forms and the presumptively infecting serovars subsist from previous reports as Australis and Autumnalis usually accompanied by the symptoms like fever, myalgia, and nausea and vomiting, jaundice like signs, conjuctival suffusion and haemorragic conditions[20]. Traditionally, leptospirosis has been considered as a febrile illness. However, they generally remain undiagnosed or are misdiagnosed due to perplexing signs and symptoms, that too under such flood menace marking out the infection becomes extremely complicated unless the disease is suspected in the presence of suggestive epidemiological information. Apart from the environmental risk factors suitable for survival of leptospires, a large population of intermediary hosts like rodents, cattle, dogs and cats which are domesticated by human and susceptible to be in more contact with population during such flood conditions can be an epidemiological niche for frequent transmission of leptospires [21]. Previously studies on human outbreaks have largely relied on serological methods to substantiate clinical cases and to define indirectly the infecting isolate. The standard serological method (MAT) provides a broad idea of serovars responsible for leptospirosis in a given geographic area in spite of the rapid methods like Leptocheck and IgM ELISA. Recently, molecular based methods involving real time PCR has been successfully used to study human outbreaks in Brazil and to characterize isolates recovered from human between 1995 and 2001 in Andaman and Nicobar Islands in India [8]. The requirements of specialized personnel skill for execution, time consuming limitations and maintenance of strains for the preparation of live antigens in laboratory are an everlasting downside of the microscopic agglutination test, although it remains as most widely used reference test. Further the knowledge of the prevalent serovars in a particular geographic area is required as it would be impossible to test with more than 200 pathogenic serovars especially in the situation of such outbreaks under flood havoc conditions. There is an emergency need for a highly sensitive and specific test for early diagnosis of leptospirosis. The sensitivity of these rapid tests usually ranges from 91% to 96.5% and specificity from 78.4% to 95.5%. Identifying leptospirosis as a cause of an outbreak of undifferentiated febrile illness among the population principally after heavy floods in Surat district and the mortality reminds us of the epidemic potential of this disease and its association with particular epidemiologic scenarios. However, the surveillance had emphasized the need for simple, improved and affordable rapid diagnostic tests with high sensitivity and specificity for early diagnosis of leptospirosis that can definitively detect individual patients and thereby tends to reduce mortality rate during the heavy flood endemic periods. The deployment of rapid molecular approaches like real time PCR can be very well considered for such endemic circumstances to efficiently overcome the difficulties tied up with basic serological methods.

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