

Short Communication

An Urban Outbreak of Leptospirosis in Mumbai, India

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SUMMARY: An outbreak of leptospirosis occurred during the rainy season in the city of Mumbai, India. Out of 169 suspected cases, 74 (43.7%) were determined serologically positive by microagglutination test (MAT) carried out with a battery of eight pathogenic serovars, while 78 (46.1%) were shown positive for IgM antibodies to leptospira by enzyme-linked immunosorbent assay. On the basis of MAT, serovar Copenhageni accounted for 66 (89.1%) out of the 74 cases admitted during the period of the outbreak. Myalgia, conjunctival suffusion, cough with hemoptysis, icterus, and oliguria were significantly more common in patients whose samples were determined positive by MAT. The presence of pulmonary signs and symptoms and renal failure were significantly associated with mortality in patients presumed to be suffering from leptospirosis.

Epidemics of leptospirosis are being increasingly reported from India (1-3), with most outbreaks occurring during the rainy season (1). An outbreak of febrile illness occurred in suburban Mumbai in the state of Maharashtra, India, in the year 2000. We investigated this outbreak and present the findings.

An outbreak of febrile disease occurred in the northern suburbs of the city of Mumbai, the capital city of the western Indian state of Maharashtra. The outbreak occurred during the rainy season in the months of August and September 2000. A total of 169 individuals were hospitalized. Acute phase blood samples were collected late during the first week of illness from all 169 patients. Convalescent samples, collected 2 to 3 weeks after the acute sample, could be obtained from 132 of these patients. Sera were separated and sent to the Microbiology Department at the Byramjee Jeejeebhoy Medical College, Pune, for carrying out tests for leptospirosis. All samples were tested for the presence of antibodies to leptospira by means of the microagglutination test (MAT) and IgM-enzyme-linked immunosorbent assay (IgM-ELISA). MAT was performed with a battery of eight representative serovars of *Leptospira interrogans*; namely, Australis, Autumnalis, Bataviae, Canicola, Copenhageni, Grippityphosa, Hebdomadis, and Pomona. *Leptospira biflexa* serogroup Semarang serovar Patoc was also included in the test. The selection of these serovars used was based on the fact that they have been commonly reported from other parts of India (3-6), as data specifically from Mumbai was not available. MAT was performed according to the standard protocol (7). The criterion for a positive MAT was a fourfold rise in antibody titer in paired sera, seroconversion to a titer of at least 200, or a single serum sample showing a titer of 200 or more. In situations where multiple serovars reacted with serum samples, the one that reacted most strongly was considered as the infecting serovar. IgM-ELISA using leptospira antigen attached to the polystyrene surface of microwell test strips was performed according to the manufacturer's (Pan-Bio, Brisbane, Australia)

instructions. Absorbance was read at 450 nm and readings were interpreted in terms of Panbio units calculated as per the instructions. Samples were recorded as positive if the number of Panbio units was more than 11. Positive control serum, negative control serum, and cut-off calibrator were provided by the manufacturer and their absorbances were used for calculation of Panbio units and for determining the validity of the test. Culture was attempted in 10 cases during the later part of the epidemic using bedside inoculation techniques as recommended (8).

Twenty-seven (15.9%) out of 169 acute phase sera were determined positive by MAT on the basis of a titer of 200 or more while 62 (36.6%) samples were recorded as positive and 7 (4.1%) as equivocal by IgM-ELISA. On the basis of these preliminary observations, the public health authorities were notified of the suspected outbreak of leptospirosis. Tests carried out on both acute as well as convalescent phase sera determined that 74 samples were positive by MAT using pathogenic serovars and 78 by IgM-ELISA. Thus, 47 samples were shown positive by MAT on the basis of tests on paired sera despite having a titer of less than 200 in the acute phase sera. Eleven (23.4%) out of these 47 showed seroconversion to a titer of at least 200, and 36 (76.5%) had a fourfold rise in titer. Twenty-four (88.8%) out of 27 samples that were presumed to be positive by MAT on the basis of acute phase sera also showed a fourfold rise in antibody titer in the convalescent sera, and in the remaining three cases convalescent samples could not be obtained. Sixty-six (89.1%) out of 74 samples shown positive by MAT showed the highest titer to serovar Copenhageni, six (8.1%) to Canicola and two (2.7%) to Bataviae. Cross reactivity between serovars was observed in the acute phase sera. Fifty-six (75.6%) out of 74 MAT-positive sera had titers ranging from 1,600 to 6,400, with the highest titer obtained being 25,600. Reactivity to serovar Patoc was observed in 60 (81%) out of the 74 samples. All samples that were not reactive to the pathogenic serovars were also not reactive to Patoc. Table 1 shows a comparison between the results of MAT and those of IgM-ELISA. *Leptospira* could not be cultured from any of the 10 cases. The clinical features of the cases are summarised in Table 2. Eleven (14.8%) out of 74 patients died.

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Table 1. Results of IgM-ELISA as compared to MAT based on both acute and convalescent phase sera

Serological Test	MAT Positive	MAT Negative	Total
IgM-ELISA Positive	67	11	78
IgM-ELISA Negative	7	84	91
Total	74	95	169

Sensitivity of IgM-ELISA: 90.5% (67/74)
 Specificity of IgM-ELISA: 88.4% (84/95)
 Positive Predictive Value: 85.8% (67/78)
 Negative Predictive Value: 92.3% (84/91)

Outbreaks of leptospirosis have recently occurred in various parts of the country and leptospirosis has now been recognized as an emerging infection. In tropical countries, incidences of leptospirosis peak during the rainy season (9). The outbreak that we have reported occurred during the monsoon season in the western part of India. Pulmonary symptoms including cough and hemoptysis were observed in 26 (35.1%) out of 74 patients. Pulmonary manifestations of leptospirosis are being increasingly reported from India (1). Icterus, oliguria, pulmonary symptoms, and conjunctival suffusion were more commonly associated with patients who were positive by MAT as compared to those who were negative (Table 2). Nine (81.8%) out of 11 patients who died had pulmonary symptoms and eight (72.7%) had oliguria. In contrast, pulmonary manifestations were observed in only 17 (26.9%) and renal manifestations in 13 (20.6%) out of 63 survivors. Pulmonary and renal manifestations are known to be indicators of poor prognosis (10,11).

Rapid diagnosis of leptospirosis is therapeutically important and is effective only when administered early in the course of illness (12). In our study, ELISA could suggest a diagnosis of leptospirosis on the basis of acute phase sera in 62 cases as compared to 27 cases shown positive by MAT. The ELISA that we have used detects only IgM antibody. IgM antibodies appear during the first week of illness and its detection has been shown to be more sensitive than MAT during the acute phase of the disease (9). In 11 patients in our study, IgM-ELISA was positive but the diagnosis could not be confirmed by MAT possibly because of a sluggish agglutinin response. Delayed serological response in agglutinin titer has been shown to occur in leptospirosis (13).

Based on MAT, serovar Copenhageni (serogroup Icterohemorrhagiae) was presumably responsible for the majority

of cases in the present study. Although seroepidemiological data from Mumbai are not available, we had earlier reported an outbreak of leptospirosis due to serovar Copenhageni from coastal areas of Maharashtra (2). In another study on febrile patients, leptospira belonging to serovars Autumnalis and Copenhageni were reported as the two most common serovars responsible for leptospirosis in and around Pune and in western Maharashtra (14). However, pulmonary manifestations were not noted in these earlier reports. *Leptospira* could not be cultured in the 10 cases where it was attempted. This could be due to administration of antibiotics by local practitioners before patients were referred to hospitals.

We recommend the use of serological tests such as IgM-ELISA for rapid diagnosis of a suspected outbreak, although MAT continues to be the definitive serological investigation regarding leptospirosis. MAT is also useful for identification of the responsible serovar in cases that are culture-negative. Knowledge of the infecting serovar can help in tracing the animal reservoir as different animals serve as maintenance hosts for distinct serovars (9). This would help in instituting appropriate control measures and help prevent the spread of epidemic.

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Table 2. Various clinical features of the cases during the outbreak

Clinical features	MAT positive (n = 74)		MAT negative (n = 95)		P value
	Number	%	Number	%	
Fever	74	100	93	97.8	0.2
Headache	68	91.8	87	91.5	0.9
Myalgia	50	67.5	37	38.9	<.01*
Conjunctival suffusion	26	35.1	5	5.2	<.01*
Cough with hemoptysis	26	35.1	4	4.2	<.01*
Icterus	25	33.7	1	1	<.01*
Oliguria	21	28.3	7	7.3	<.01*
Lymphadenopathy	11	14.8	7	7.3	0.11
Arthralgia	9	12.1	16	16.8	0.39
Rash	9	12.1	8	8.4	0.42
Meningeal signs	0	0	2	2.1	0.2
Mortality	11	14.8	2	2.1	<.01*

* Significant difference as per chi square test

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