Short Report: Leptospirosis as a Cause of Fever in Urban Bangladesh

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Abstract. We tested paired sera from 584 febrile persons in an low-income urban community in Bangladesh for evidence of *Leptospira* infection. A total of 8.4% of the persons met criteria for definite or probable infection. Persons with leptospirosis were older than those with undifferentiated fever in this population. The dominant infecting serogroups in Bangladesh differed from serogroups commonly reported in nearby regions.

Leptospirosis is a widespread zoonotic disease that is increasingly being recognized in urban settings within developing countries.^{1–3} Human infection results from exposure to pathogenic spirochetes of the genus *Leptospira*, often by skin contact with contaminated water or soil.^{4,5} In low-income urban neighborhoods, rats are important carrier mammals and excrete the organism in urine.^{6–8} Conditions of poor sanitation, flash flooding, and overcrowding may facilitate transmission of the disease.^{2,9,10} Because cases often present as a nonspecific febrile illness,^{8,11} and because no reliable, rapid, and readily-available diagnostic test exists, many cases go unrecognized.^{4,10} Serum polymerase chain reaction or blood culture can provide a definitive diagnosis,¹² but are not widely available. Tests for the presence of genus-specific IgM,¹³ which develops within the first week of infection and persists for months,¹⁴ may be used for screening, and a microscopic agglutination test (MAT) can provide greater specificity along with some indication of the infecting serovar.¹⁵

Leptospirosis has been described in several regions of India,^{16–19} but data on the epidemiology of leptospirosis in Bangladesh are limited. One report documents leptospirosis in hospitalized patients during a dengue outbreak in 2000, at which time 18% of dengue-negative febrile patients at two Dhaka hospitals were positive for leptospirosis by PCR.²⁰ In a 1994 serosurvey in rural Bangladesh, a high prevalence of low-level MAT reactivity was identified among jaundiced febrile patients and among healthy controls.²¹ In this study, we evaluated the prevalence of leptospirosis among febrile outpatients in a low-income area of Dhaka, Bangladesh.

In 2001, the International Center for Diarrheal Disease Research in Dhaka, Bangladesh conducted surveillance for febrile illness in Kamalapur, a low-income neighborhood in Dhaka. Once per week throughout that year, field research assistants screened representative households. The assistants referred all persons with at least three days of fever (axillary temperature $\geq 38^{\circ}\text{C}$), and children less than five years of age with fever of any duration, to a field clinic. Additionally, some persons with less than three days of fever self-referred to the clinic. Upon enrollment, a study physician collected clinical data, conducted a physical examination, and obtained blood for routine bacteriologic culture²² and for serologic testing.

Patients were instructed to return at least one week later to provide a convalescent-phase serum sample. Study workers obtained written informed consent from all participating households, and the protocol was reviewed and approved by the research and ethical review committees of the International Centre for Diarrhoeal Disease Research, Bangladesh.

Convalescent-phase serum samples were screened for *Leptospira*-specific IgM by microplate enzyme-linked immunosorbent assay (ELISA) (PanBio Diagnostics, Brisbane, Queensland, Australia) at the Centers for Disease Control and Prevention in Atlanta, Georgia. For patients with a positive or equivocal convalescent-phase IgM result, MAT was then performed on the convalescent-phase serum sample and on the associated acute-phase serum sample when available. We used a standard MAT method^{23,24} with a broad battery of 39 *Leptospira* serovars representing all known pathogenic serogroups. Homologous hyperimmune rabbit serum was included in each MAT run for quality control, and samples were first screened at a 1:100 dilution against all serovars, after which serum samples were titrated by further, serial, two-fold dilutions against the serovars that had initially yielded positive results, with results checked for consistency across dilutions.

Because serologic criteria for the diagnosis of leptospirosis vary widely between studies,²⁵ we used two separate case definitions. Patients with an MAT titer ≥ 100 for any serovar on either acute-phase or convalescent-phase serum samples were categorized as having probable leptospirosis. This high-sensitivity criterion is commonly used to define cases among ill patients in endemic or outbreak settings.^{26,27} Patients with either a single MAT titer ≥ 800 for any serovar at either phase, or a serovar-specific four-fold increase from acute phase to convalescent phase with a minimum convalescent titer of 400, were categorized as definite leptospirosis. This definition is consistent with the strictest published serologic definitions.^{26,28,29} Dengue MAC ELISA (PanBio Diagnostics) was also performed on collected serum samples.

Statistical analyses were performed by using Intercooled Stata version 9.1 (Stata Corp., College Station, TX). Diagnostic groups were compared using equal-variance unpaired *t*-tests and Pearson's chi-square tests, with two-sided *P* values reported. Forward stepwise logistic regression was performed for the multivariate analysis, with an inclusion cutoff criterion of *P* ≤ 0.2 .

Eight hundred seventy-eight febrile persons were identified in the Kamalapur surveillance community from January 1 through December 31, 2001. Convalescent-phase serum samples from 584 persons (67%) collected between 9 and 103 days (median = 22 days) after onset of illness were available for

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Leptospira-specific IgM ELISA testing. Patients with missing convalescent-phase sera tended to be older (mean age = 12.0 years versus 10.1 years; $P = 0.02$) and more frequently male (62% versus 49%; $P = 0.001$) than the included population.

Sixty-two convalescent-phase serum samples (11%) were IgM positive, and 16 (3%) were equivocal; these 78 samples were further tested by MAT. Seven acute-phase serum samples corresponding to positive or equivocal IgM results had insufficient serum for MAT testing. Samples from these patients were included in the present study, and the decision of whether they met the leptospirosis case definition was made on the basis of the convalescent phase titer alone. Of the 78 samples identified for MAT testing, 13 (2.2% of all persons screened) met MAT criteria for definite leptospirosis: seven by having a ≥ 4 -fold increase in *Leptospira* MAT titer, two by having convalescent-phase titers of 800 with unavailable acute-phase serum samples, and four by having high acute-phase and convalescent-phase titers (range = 1,600–12,800). An additional 36 of the 78 cases identified by IgM for further testing (6.2% of all persons screened) met criteria for probable leptospirosis. The remaining 29 IgM-positive or IgM-equivocal samples were negative by MAT.

Serogroups identified by MAT among patients with probable and definite leptospirosis are shown in Table 1. Seropositivity to the Sarmin serogroup was most common. However, patient serum samples frequently showed cross-reactivity with multiple serogroups. Although it would be desirable to identify the infecting serovars based on MAT data, existing literature suggests that such determinations are inaccurate because of frequent cross-reactivity.^{26,30}

Some clinical features differed between patients with leptospirosis and patients with undifferentiated fever (Table 2).

TABLE 1

Leptospirosis cases identified by microscopic agglutination test in febrile patients in Kamalapur, Bangladesh with positive or equivocal IgM results, by serogroup*

Serogroup† (serovar[s] included in MAT)	No. of cases meeting case definition for each serogroup (range of MAT titers within serogroup)	
	Probable leptospirosis cases (n = 36)	Definite leptospirosis cases (n = 13)
Sarmin (Sarmin, Rio, Waskurin, Machiguenga)	22 (100–400)	7 (400–6,400)
Mini (Georgia, Sari)	9 (100–200)	4 (400–12,800)
Australis (Australis, Bratislava)	6 (100–400)	6 (800–12,800)
Louisiana (Lanka)	7 (100–400)	4 (1600–25,600)
Icterohaemorrhagiae (Icterohaemorrhagiae, Mankarso, Copenhagen)	2 (100–200)	4 (800–12,800)
Autumnalis (Autumnalis, Bangkinang, Fort Bragg, Butembo)	3 (100–400)	3 (800–3,200)
Shermani (Shermani)	5 (100–200)	1 (400)
Javanica (Javanica, Poi)	2 (100)	2 (800–3,200)
Djasiman (Djasiman)	2 (200)	1 (400)
Pyrogenes (Pyrogenes, Alexi)	1 (100)	1 (12,800)
Sejroe (Hardjo, Wolffi)	2 (200)	0
Cynopteri (Cynopteri)	0	1 (1,600)
Celledoni (Celledoni)	0	1 (800)
Panama (Panama)	1 (200)	0

* MAT = microscopic agglutination test. Column totals exceed n values because many patients had positive MAT results for more than one serogroup.

† Additional serogroups (serovars) tested that yielded no positive MAT results: Ballum (Ballum), Bataviae (Bataviae), Canicola (Canicola), Grippotyphosa (Grippotyphosa), Hebdomadis (Hebdomadis, Borincana), Hurstbridge (Hurstbridge), Manhao (Manhao 3), Pomona (Pomona), Ranarum (Ranarum), and Tarassovi (Tarassovi), and Genomospes 1 serovar Sichuan.

Persons with leptospirosis were significantly older than those with undifferentiated fever, and they were more likely to report chest pain. Rhinorrhea, abnormal breath sounds, and pharyngitis were more common in persons with undiagnosed, non-leptospiral febrile illness. Multivariate analysis showed that age in years (odds ratio = 1.04 per additional year, 95% confidence interval = 1.02–1.06, $P < 0.001$) and chest pain (odds ratio = 15.5, 95% confidence interval = 3.5–70.0, $P < 0.001$) were independent predictors of leptospirosis, although chest pain was an infrequent finding.

Of the 49 probable and definite leptospirosis cases, 36 occurred during January through May, a period that spans late winter to the start of the monsoon season in this region. We detected no geographic clustering of leptospirosis cases within the Kamalapur surveillance area.

Febrile patients were additionally evaluated for dengue, enteric fever, and bloodstream infection. There was no overlap between the diagnoses of dengue, enteric fever, and leptospirosis. However, two patients with probable leptospirosis had positive blood cultures: one for *Acinetobacter* species and one for group D *Streptococcus*, the latter of which may have represented contamination.

TABLE 2

Demographic and clinical features of patients with leptospirosis and with undiagnosed fever in Kamalapur, Bangladesh, 2001*

Feature	Probable or definite leptospirosis (n = 49)	Undiagnosed fever† (n = 500)	P
Demographics			
Sex, male, no. (%)	20 (41)	251 (50)	0.2
Age, years, mean $\pm$ SD	17.8 $\pm$ 13.1	9.2 $\pm$ 11.0	< 0.001
Symptoms			
Fever duration, days, mean $\pm$ SD)	3.7 $\pm$ 2.4	3.5 $\pm$ 1.8	0.6
Headache	40/46 (87%)	247/315 (78%)	0.2
Abdominal pain	18/46 (39%)	116/359 (32%)	0.4
Myalgia	31/46 (67%)	186/315 (59%)	0.3
Watery stool	10/49 (20%)	97/500 (19%)	0.9
Chest pain	5/49 (10%)	3/500 (1%)	< 0.001
Cough	33/49 (66%)	365/500 (73%)	0.4
Dyspnea	4/49 (8%)	40/500 (8%)	0.97
Rhinorrhea	25/49 (51%)	328/500 (66%)	0.04
Sore throat	3/49 (6%)	40/500 (8%)	0.6
Weakness	1/49 (2%)	7/500 (1%)	0.7
Rash	1/49 (2%)	7/500 (1%)	0.7
Bleeding anywhere	2/49 (4%)	14/500 (3%)	0.6
Vomiting	11/49 (22%)	84/500 (17%)	0.3
Anorexia	36/49 (73%)	397/500 (79%)	0.3
Neck stiffness	2/49 (4%)	5/481 (1%)	0.07
Retroorbital pain	5/49 (10%)	30/500 (6%)	0.3
Urinary symptoms	10/49 (20%)	59/500 (12%)	0.08
Physical findings			
Temperature, °C, mean $\pm$ SD	38.9 $\pm$ 0.7	38.9 $\pm$ 0.9	0.8
Abnormal breath sounds	6/49 (12%)	129/500 (26%)	0.04
Abdominal tenderness	1/49 (2%)	11/500 (2%)	0.9
Conjunctival suffusion	1/49 (2%)	7/500 (1%)	0.7
Pharyngitis	7/49 (14%)	140/500 (28%)	0.04
Altered mental status	5/49 (10%)	49/500 (10%)	0.9
Lymphadenopathy	10/49 (20%)	124/500 (25%)	0.5
Lymph node tenderness	2/49 (4%)	16/500 (3%)	0.7

* A denominator less than the n value for an individual symptom indicates the recording of an unknown response, usually in young children. These responses were treated as missing data in the statistical analysis.

† Patients with a confirmed diagnosis of dengue fever or enteric fever were excluded.

We report the findings of a community-based study of fever etiology in a low-income area of Dhaka, Bangladesh, with an emphasis in this report on the diagnosis of leptospirosis. Based on serologic testing, we found that leptospirosis accounted for between 2% and 8% of outpatient fever episodes. These results suggest that health care providers in Bangladesh should consider leptospirosis as a cause of acute febrile illness in outpatients and be prepared to initiate appropriate antibiotic therapy when indicated.³¹ We found only limited differences in clinical features between patients with leptospirosis and those with other causes of undifferentiated fever in our study population. However, with clinical data from more cases, it may be possible to develop a leptospirosis probability scoring system to guide diagnostic testing or empiric therapy in Bangladesh. The concentration of leptospirosis cases in the early, dry part of the year differs from patterns observed elsewhere,^{9,32} but may be attributable to the year-round persistence of wet conditions in low-lying areas in Dhaka and stagnation of standing water during the dry season.

Patients in this study population reacted to a broad range of *Leptospira* serogroups. Several patients had positive MAT results for serogroups previously identified as common in rural Bangladesh (Australis, Icterhaemorrhagiae, Cynopteri),²¹ but overall, the reactive serogroups in our study population differ from the serogroups that have been reported elsewhere in southern^{16–19} or Southeast³³ Asia. In particular, the two most frequently reactive serogroups among our study subjects, Sarmin and Mini, have not been reported elsewhere in the region. Additionally, only 49 out of 78 patients with IgM against *Leptospira* had positive results by MAT, despite the use of a broad panel of serovars. This finding suggests that unidentified serovars could be circulating in Bangladesh, and we may consequently have underestimated the prevalence of leptospirosis in our study population. This observation also highlights the need to further define the circulating *Leptospira* serovars in Bangladesh to include them in locally-relevant diagnostic tests.

Our study has a number of limitations. Convalescent-phase serum samples were not available for nearly one-third of patients with fever. The clinical features of these persons resembled those of the overall febrile patient population, but the excluded persons tended to be older. Because leptospirosis affected all age groups and other febrile illnesses were more common in children than adults, the loss of many adults to follow-up may have led us to underestimate the prevalence of leptospirosis in our study population. Also, we studied only one low-income community in Dhaka; the prevalence of leptospirosis may be different in other parts of Dhaka or Bangladesh. Finally, serologic diagnosis of leptospirosis is less specific than culture-based testing, and it is possible that some low-titer positive serologic results relate to previous exposure rather than current illness.

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