

Seroprevalence of leptospirosis in a rural flood prone district of Bangladesh

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SUMMARY

Leptospirosis is a worldwide zoonotic disease. In the present investigation, a total of 89 human sera from a flood prone district of Bangladesh was screened by a one-point microcapsule agglutination test (MCAT). MCAT-positive and -doubtful sera were further tested by microscopic agglutination test (MAT) against 16 reference serovars of *Leptospira interrogans*, and the antibody titres determined. In MCAT, 34 sera were positive and 22 were doubtful. Among those positive and doubtful sera, 33 and 20, respectively were tested by MAT. Thirty-four out of 53 MCAT-screened samples were MAT-positive. The titres ranged from 20 to 1600 with antibodies to serovars *copenhageni*, *australis*, *cynopteri* and *icterohaemorrhagiae* being the most prevalent. Eleven MCAT-positive samples failed to react with any strains used by MAT, suggesting the presence of new or untested serovars. Among the MAT-positive samples, the presence of antibody against two or more serovars was more common than that of a single serovar. The present study suggests that rural people in Bangladesh are at high risk to leptospiral infection.

Leptospirosis, caused by the pathogenic members of the genus *Leptospira*, is an important zoonotic disease. The species consists of over 200 serological variants or serovars with world-wide distribution including Southeast Asia [1-8]. Clinical illness varies in severity from a mild non-specific febrile illness to a severe fatal form with hepatorenal failure [1]. Bangladesh experiences flooding almost every year. The geographical location, climatic conditions and rich fauna seem to be suitable for the survival of leptospires. Leptospirosis, however, has not been reported in this country although it has been reported in India [2, 3] and in Pakistan [4]. In the present study, we determined the leptospiral antibody titres

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in the sera of 89 humans living in a flood-prone river-side district of Bangladesh (Fig. 1).

The sera were collected between June 1992 and July 1992 from 58 individuals with a history of fever persisting for more than one week, or jaundice, or both. Thirty-one samples were collected from individuals without such a history as controls. Age and sex were recorded in all cases. Two drops of 10% sodium azide were added to the serum to prevent bacterial multiplication and brought to our laboratory in Japan. A one-point microcapsule agglutination test (MCAT) which has shown the promising results [8–10] was used as a screening test. All sera were inactivated at 56 °C for 30 min and the MCAT performed [9] using a commercial kit (Japan Lyophilization Co., Japan). The results were scored as follows; positive (+), when the agglutination pattern slipped or covered the entire bottom of the tube, or was apparently larger than negative control; doubtful (\pm), when the agglutination pattern was slightly larger than negative control; and negative (–), if no agglutination was observed.

Among the 58 samples from people with a history of clinical illness, 19 sera were positive and 19 doubtful in the MCAT. Similarly, 15 and 3 of 31 control samples were positive and doubtful respectively (Table 1). This difference was not significant (chi-square test for independence, $P > 0.05$) and hence the analyses of other subsequent experiments were carried out combining both sets of samples.

The prevalence of MCAT-positive sera was analysed by age and sex. There was a tendency for low positivity in people aged more than 51 years ($P = 0.078$, data not shown), probably because they had retired from farming. It should be noted that one positive and two doubtful sera were obtained from the people residing in the riverside area. It has been reported that leptospiral antibodies are more prevalent in males than in females [1, 7] although this may not always be the case [11]. In this study, there was no difference between sexes (data not shown), possibly because in rural areas of Bangladesh, men and women are engaged equally in working in the fields. Moreover, all the people in this district are likely to be exposed to the flood water in most years.

Most of the MCAT-positive and -doubtful sera were obtained from the volunteers who live within 2 km from the banks of Jamuna or its branch river (Fig. 1). We measured the distance from each volunteer's residence to the nearest river. Statistical analysis revealed that the distances in the MCAT-positive and -doubtful groups are significantly shorter than those in the MCAT-negative group (Kruskal–Wallis test, $P < 0.01$). When the flood water recedes, all the waste materials are washed away and are deposited in the river. The river side people have contact with the river water for their daily life, e.g. bathing, swimming and so on. It is also well known that leptospires can remain viable in natural water for a long time [1]. So these findings suggest that the river plays an important role in the survival and spread of leptospires in Bangladesh.

To determine which serovars were involved the 33 MCAT-positive and 20 MCAT-doubtful sera were subjected to the MAT [12], using 16 serovars of *Leptospira interrogans* (Table 2) grown in Korthof medium containing 10% heat-inactivated rabbit sera and incubated for 3–10 days at 28 °C [1]. The titre was expressed as the highest dilution of antiserum with 50% microscopic agglutination; titres of 20 or more were considered positive. Thirty-four MCAT-

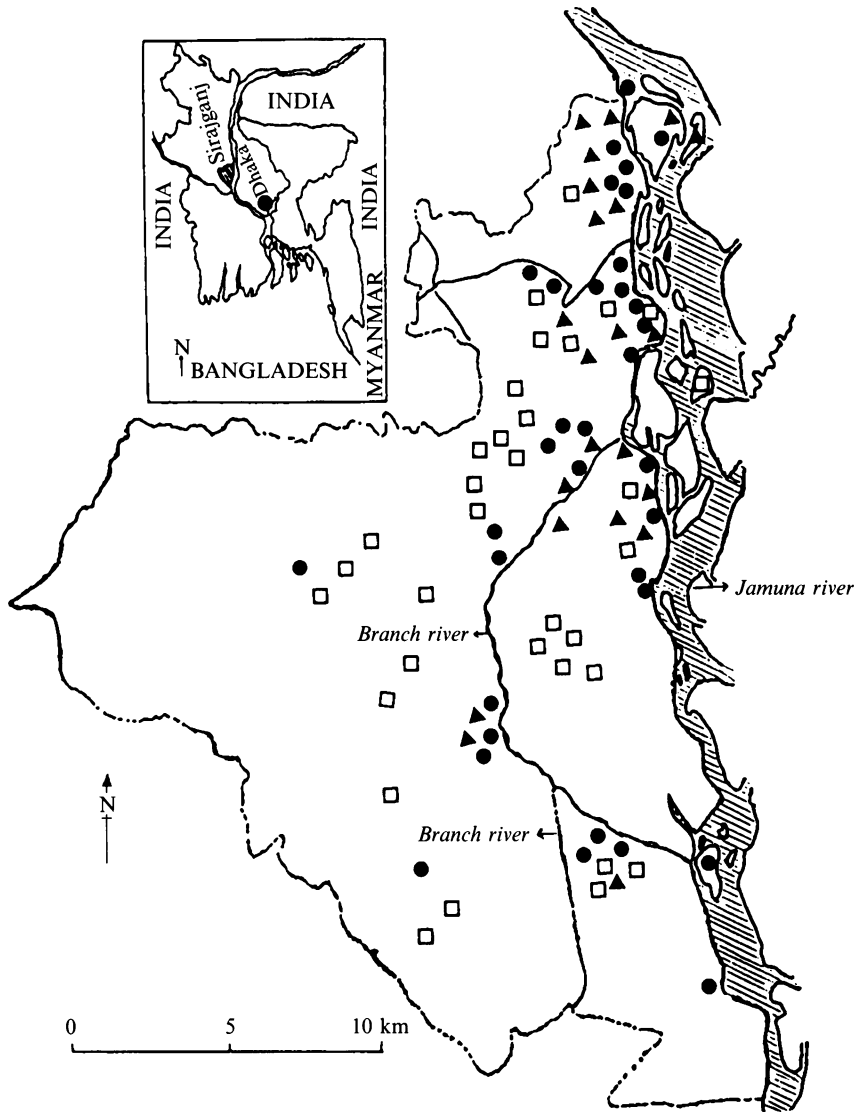


Fig. 1. Location of the residence of volunteers who donated blood. ●, MCAT positive sera; ▲, MCAT doubtful sera; □, MCAT negative sera. Inset: Map of Bangladesh showing the location of Sirajganj where volunteers reside.

screened sera reacted with one or more of the serovars. Serovar *copenhageni* reacted with 21% of the sera followed by *australis*, 19%; *cynopteri*, 18%; *icterohaemorrhagiae*, 13%; *hardjo*, 7%; *wolffi*, 6%; *hebdomadis*, 5%; *grippityphosa*, 4%; *canicola*, 2%; *pomona*, 2%; *javanica*, 1% and *castellonis*, 1%. The highest titre was 1600. In contrast, serovars *autumnalis*, *bataviae*, *pyrogenes* and *tarassovi* did not react with any sera. Among the 34 MAT-positive sera of MCAT-screened sera, 11 sera (32%) reacted with a single serovar and the other 23 sera (78%), with 2–8 serovars (data not shown). The presence of multiple antibodies seems to be common in both human and other mammals [7, 11, 13], possibly as a

Table 1. *Prevalence of leptospiral antibody in human sera of Bangladesh determined by a one-point microcapsule agglutination test (MCAT)**

Sample	No. of sera			
	Tested	Positive (%)	Doubtful (%)	Negative (%)
Study†	58	19 (32.7)	19 (32.7)	20 (34.5)
Control‡	31	15 (48.4)	3 (9.7)	13 (41.9)
Total	89	34 (38.2)	22 (24.7)	33 (37.1)

* MCAT consists of reagent A and B; reagent A contains microcapsules coated with serovars *australis*, *autumnalis* and *hebdomadis*; reagent B contains microcapsules coated with serovars *canicola*, *icterohaemorrhagiae* and *pyrogenes*. Positive, sera agglutinated with reagents A or B or both; doubtful, sera giving a doubtful agglutination with reagents A or B or both; negative, no agglutination with either reagent.

† Sera from people with a history of prolonged fever or jaundice or both.

‡ Sera from people with no such a history.

Table 2. *Distribution of sera which were positive (33 samples) or doubtful (20 samples) to a one-point microcapsule agglutination test according to the titres to different serovars as measured by a microscopic agglutination test*

Serovar (strain)	No. of sera with titre of					Total no. (%)
	20	40	80	160	≥ 320	
<i>copenhageni</i> (Shibaura)	2	4	7	4	0	17 (20.5)
<i>australis</i> (Ballico)	7	2	5	1	1	16 (19.3)
<i>cynopteri</i> (3522-C)	2	2	5	5	1	15 (18.1)
<i>icterohaemorrhagiae</i> (RGA)	1	5	3	1	1	11 (13.2)
<i>hardjo</i> (Hardjoprajitno)	0	1	0	1	4	6 (7.2)
<i>wolffi</i> (3705)	0	2	1	0	2	5 (6.0)
<i>hebdomadis</i> (Hebdomadis)	0	0	3	0	1	4 (4.8)
<i>grippotyphosa</i> (Moskva V)	0	2	1	0	0	3 (3.6)
<i>canicola</i> (Hond Utrecht IV)	1	0	0	1	0	2 (2.4)
<i>pomona</i> (Pomona)	1	0	0	1	0	2 (2.4)
<i>javanica</i> (V.B46)	0	0	1	0	0	1 (1.2)
<i>castellonis</i> (Castellon 3)	1	0	0	0	0	1 (1.2)
<i>autumnalis</i> (Akiyami A)	0	0	0	0	0	0
<i>bataviae</i> (v. Tienen)	0	0	0	0	0	0
<i>pyrogenes</i> (Salinem)	0	0	0	0	0	0
<i>tarassovi</i> (Mitis Johnson)	0	0	0	0	0	0

result of multiple infections or cross reaction between different serovars. Interestingly, this study revealed that *cynopteri*, which has not been reported as the predominant serovar in any country, is one of the predominant serovars in Bangladesh. In particular, three samples showed positive reaction for serovar *cynopteri* alone. This was not due to a cross-reaction, however, since the anti-*cynopteri* antibody was not absorbed by the other serovars.

Twenty-two out of 33 MCAT-positive sera reacted with the serovars of *L. interrogans* in the MAT (data not shown), while there were 12 sera with a positive reaction in the MAT among 20 MCAT-doubtful sera. The findings that 11 MCAT-positive sera did not react with any serovar in MAT indicate the presence of serovars not included among reference strains, since it has been reported that MCAT is genus specific rather than species specific [9]. Moreover, 6 of 34 MAT-

positive sera reacted with only one serovar (*copenhageni*, *cynopteri* and *pomona*) not included in MCAT kit used (data not shown). These findings suggest that MCAT is a useful tool for the screening of antibodies in the epidemiological study of leptospirosis in situations when the predominant serovars are unknown. On the other hand, 12 sera showed positive reaction in MAT among 20 MCAT-doubtful sera. This was possibly due to the presence of serovars which were not coated to MCAT microcapsules or due to the late infection with the coated serovars. These results also indicate that new combinations of serovars, including the serovar *cynopteri*, should be coated to MCAT reagents in Bangladesh since this could increase the detection-rate of leptospiral antibodies.

The present study showed that exposure to leptospire occurred frequently among the rural population of Bangladesh, suggesting that the disease has been under-diagnosed in this country. Clearly further studies are required to establish maintenance host for the different serovars.

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