Short Communication

Serological and Genetic Analysis of Leptospirosis in Patients with Acute Febrile Illness in Kandy, Sri Lanka

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SUMMARY: Leptospirosis has emerged as an important infectious disease in Sri Lanka and little information is available on circulating leptospiral species and serogroups in this country. Therefore, we studied circulating leptospiral species and serogroups in patients with acute febrile illness using polymerase chain reaction and the microscopic agglutination test, respectively. Anti-leptospiral antibodies were detected in 26 of 107 serum samples studied (24.3%). The predominant reacting serogroups were Sejroe (9/26, 34.6%) and Icterohaemorrhagiae (5/26, 19.2%). Leptospiral DNA was detected in 3 of the 107 serum samples. The deduced leptospiral species were Leptospira interrogans and L. kirschneri (2 and 1 samples, respectively). These results confirm the existence of a wide array of leptospiral species and serogroups in Sri Lanka and would help to thoroughly elucidate the epidemiology of leptospirosis in this country.

Leptospirosis is caused by pathogenic strains of Leptospira. It is a globally prevalent zoonosis affecting humans in rural and urban settings in both industrialized and developing countries (1-3). Transmission of Leptospira to humans occurs mainly through contact with water or soil contaminated by the urine of infected animals (4). Leptospirosis has become an important public health problem in Asia and Latin America (3). In Sri Lanka, the number of reported cases of leptospirosis has increased recently. One to two thousand cases were recorded annually in the past 10 years and 7,421 cases (incidence rate 36.7/100,000 population) and 207 deaths (case-fatality rate, 2.8%) were reported in 2008 (5). However, most of these cases were diagnosed only clinically. Even in confirmed cases, serological diagnosis is carried out only by the microscopic agglutination test (MAT) using a saprophytic leptospiral strain (5,6). Consequently, the country lacks information on circulating leptospiral species and serogroups (7). Therefore, this study attempted to identify circulating leptospiral species and serogroups in patients with acute febrile illness who fulfilled the case-definition criteria for leptospirosis using polymerase chain reaction (PCR) and MAT, respectively, with a battery of pathogenic leptospiral strains.

The study included patients admitted to the Teaching Hospital Peradeniya, Kandy in 2008 with acute febrile illness and who fulfilled the case definition of leptospirosis (8). Serum samples were obtained from patients on the 7th day of fever. MAT was performed for detection of anti-Leptospira antibodies in patient serum samples (4) using a battery of representative leptospiral serogroups recommended by the World Health Organization (9) (Table 1). DNA was extracted from pellets after centrifugation (13,000 g, 20 min) of each serum sample using a DNeasy Tissue Kit (Qiagen, Valencia, Calif., USA). Extracted DNA was subjected to nested PCR for detection of the Leptospira flaB gene (10) and then the nucleotide sequences of the amplicons were determined. Anti-Leptospira antibodies (reciprocal MAT titer ≥400) were detected in 26 of the 107 (24.3%) serum samples of patients with acute febrile illness (Table 1). The predominant reactive serogroups were Sejroe (9/26, 34.6%) and Icterohaemorrhagiae (5/26, 19.2%). Eight of the 26 positive samples reacted equally to multiple serovars. Fifteen of the 107 serum samples were also subjected to detection of immunoglobulin-M (IgM) by IgM dot enzyme-linked immunosassay (Dip-S-Ticks; PanBio, Baltimore, Md., USA) and the results were correlated with those of the MAT mentioned above, suggesting acute infection with Leptospira (data not shown). The age distribution of the MAT-positive cases (median, 44 years; range, 23-74) showed an equal distribution among all cases examined (median, 41 years; range, 13-80). The gender distribution showed male predominance in 24/26 (unknown, 2/26). The sex bias in leptospirosis has been well demonstrated (1-3). Among the 26 positive cases studied here, 16 (61.5%) were farmers while 8 (30.8%) did not engage in agricultural work (unknown, 2/26). Occurrence of leptospirosis has been attributed to occupational exposure, such as exposure through rice farming and other agricultural activities in rural areas of the tropics, including Sri Lanka; however, only a single study has suggested that people in Sri Lanka contracted leptospirosis through environmental exposure during daily life (2,5,7). In the present study, about 30% of the patients turned out to be infected through non-farming activities. Unfortunately, we were not able to obtain more detailed information on the occupations of our patients. In the future it will be important to identify risky activities other than agricultural tasks for the prevention of leptospirosis. Infections occurred in 14 areas in the Kandy District (data not shown), indicating that leptospirosis is an epidemic in this district.
Leptospiral flaB was detected in 3 of the 107 serum samples. The leptospiral species were deduced to be *Leptospira interrogans* and *L. kirschneri* (2 and 1 samples, respectively) by comparison of the nucleotide sequences of the flaB genes from the serum samples with those from the reference strains (Fig. 1. DDBJ/GenBank/EMBL accession nos. AB512279-AB512281). This is the first report regarding circulating leptospiral species in Sri Lanka. The number of PCR-positive samples was much lower than that for MAT. This can probably be attributed to the fact that all the patients were administered antibiotics before the collection of serum samples.

In conclusion, an epidemic of leptospirosis in Kandy, Sri Lanka was verified using laboratory diagnostic methods in this study. The information obtained should help to elucidate the epidemiology of leptospirosis in this country, such as by clarifying carrier animals and the route of infection, as well as to improve the diagnostics and preventive measures.

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**REFERENCES**