Serological study of Leptospirosis in central Nepal

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Abstract
Background: Leptospirosis, an infectious disease caused by spirochetes of the genus Leptospira, is the most widespread zoonosis in the world. Humans acquire infection through contact with the urine of infected or carrier animals, either directly or through contaminated water or soil. There are only few reports documenting the serological evidence of leptospirosis in Nepal. Thus, present study aims to determine the status of leptospirosis in central Nepal.
Material and Methods: A descriptive cross sectional study was conducted in 1266 patients suspected of leptospirosis in Chitwan Medical College Teaching Hospital for a period of two years. Blood samples collected from the patients were processed for qualitative detection of leptospiral IgM antibody by ELISA (Enzyme linked immunosorbent assay).
Results: Of the 1266 samples subjected to ELISA, seropositivity was seen in 61 samples (4.8%) with the highest rate observed in the autumn season (63.9%) followed by summer season (21.3%). Majority (39.3%) of the seropositive individuals were agricultural workers. Leptospirosis was more common in males and in the people of 16-30 years.
Conclusion: Leptospirosis is common in central Nepal. Epidemiological surveillance and laboratory capacity across the region need strengthening to determine its true prevalence in the community.
Keywords: Leptospirosis, Central Nepal, Seropositivity

1. Introduction

Leptospirosis, an infectious disease that affects humans and animals, is considered the most widespread zoonosis in the world. It is caused by spirochetes of the genus Leptospira. Pathogenic leptospires live in the kidneys of many mammalian hosts, including rodents, insectivores, and livestock. Leptospires are shed into the environment, where they can survive for several years in favorable (warm and wet) conditions. Thus, leptospirosis is particularly endemic to warm and humid tropical and subtropical regions. Humans are infected by direct contact with infected animals or indirectly by contact with a contaminated environment.

Leptospirosis is an emerging public health problem globally. An international survey conducted by the International Leptospirosis Society reported ≥350,000 cases of severe leptospirosis annually. This estimate is supported by data from an assessment of the global incidence of leptospirosis, which indicated a mean global incidence rate for leptospirosis of 5 cases/100,000 population. The frequency of leptospirosis has not been well documented in Nepal.
outbreaks of acute febrile illnesses or fever of unknown origin have been reported every year in the country, however, none of these outbreaks have been so far reported with infection from leptospirosis.

Majority of leptospiral infections are either sub clinical or result in very mild illness and recover without any complications. However, a small proportion develops various complications due to involvement of multiple organ systems, leading frequently to renal, hepatic and pulmonary dysfunction. In such patients, the case fatality ratio could be about 40% or more. Because of the protean manifestations of leptospirosis, it is often misdiagnosed and under-reported.

The laboratory diagnosis of leptospirosis, a prerequisite for treatment, is usually achieved either by isolation of the causative organisms or by serological evidence indicating recent infection. Isolation of organisms from clinical samples is confirmatory, but a positive culture result may take up to 6 weeks and therefore it doesn’t contribute to early diagnosis. The microscopic agglutination test (MAT) is the reference test for diagnosis and detects antibodies at serovar levels. However, the test is time consuming, requires the use of paired sera, result interpretation is possible only by experts, can show low sensitivity and involves the use of a battery of at least 12–25 leptospires belonging to different serogroups prevalent in that region. The maintenance of stock cultures and use of live organisms creates a risk of laboratory-acquired infection. Several methods have been developed for use in diagnosis of leptospirosis as an alternative to MAT, of which IgM ELISA is the most promising and detects genus-specific antibodies. The present study aims to determine the status of leptospirosis in central Nepal using IgM ELISA.

2. Material and Methods

A descriptive cross sectional study was conducted for a period of two years (from October 2010 to September 2012) at Chitwan Medical College Teaching Hospital, Bharatpur-10, Chitwan, Nepal. During the study period, blood sample was collected once from each of 1266 leptospirosis suspected patients after a week of onset of their febrile illness and processed for qualitative detection of leptospiral IgM antibody by ELISA (Enzyme linked immunosorbent assay).

2.1. Ethical issues

Ethical clearance was obtained from the Institutional Review Committee (IRC) of Chitwan Medical College Teaching Hospital. An informed consent was taken from the individual patient before collection of blood sample.

2.2. Sample collection and storage

About 2 to 3 ml of blood was collected by venipuncture from the patients using strict aseptic precautions. It was allowed to clot at room temperature (20-25ºC) and then centrifuged according to the guideline of Clinical and Laboratory Standards Institute (CLSI). The serum was separated as soon as possible and refrigerated (2-8ºC) until tested.

2.3. Detection of IgM by ELISA (Panbio, Inverness Medical, Australia)

Panbio Leptospira IgM ELISA is qualitative detection of IgM antibodies to Leptospira in serum. The ELISA test is reported to have a sensitivity of 100% and a specificity of 93% in the diagnosis of acute leptospirosis infection. Sera prepared from the collected blood samples were subjected to ELISA and presence of leptospiral IgM antibodies was detected.

The assay relied on the following principle: serum containing antibodies to Leptospira antigen, when present, combined with Leptospira antigen attached to the polystyrene surface of the microwells. Residual serum was removed by washing and peroxidase conjugated anti-human IgM was added. The microwells were washed and a colourless substrate system, tetramethylbenzidine / hydrogen peroxide (TMB Chromogen) was added. The substrate was hydrolysed by the enzyme (horse radish peroxidase, HRP) and the chromogen changed to a blue colour. After stopping the reaction with acid (phosphoric acid), the TMB became yellow. Colour development was indicative of the presence of IgM antibodies to Leptospira in the test sample. The absorbance of each well was, further, read within 30 minutes by spectrophotometer at a wavelength of 450 nm.

3. Results

Of a total of 1266 blood samples subjected to ELISA for leptospiral IgM antibody, positivity was seen in 61 samples (4.8%). Among 61 positive cases, majority were (39.3%) were agricultural workers followed by housewives (19.7%) [table 1].
Table 1: Occupation wise distribution of leptospiral IgM antibody positive cases

<table>
<thead>
<tr>
<th>Occupation</th>
<th>No. (%) of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agriculture workers</td>
<td>24 (39.3)</td>
</tr>
<tr>
<td>Housewives</td>
<td>12 (19.7)</td>
</tr>
<tr>
<td>Students</td>
<td>11 (18.1)</td>
</tr>
<tr>
<td>Office workers</td>
<td>6 (9.8)</td>
</tr>
<tr>
<td>Labourers</td>
<td>5 (8.2)</td>
</tr>
<tr>
<td>Businessmen</td>
<td>3 (4.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>61 (100)</strong></td>
</tr>
</tbody>
</table>

The highest number (63.9%) of leptospiral cases was seen in the autumn season while the lowest number (1.7%) in spring season (figure 1).

Figure 1: Seasonal distribution of leptospiral IgM antibody positive cases

Males (35/61, 57.4%) were more commonly affected than females (26/61, 42.6%) and majority (39.3%) of the positive cases were in the age group of 16-30 years followed by 31-45 years (24.6%) [table 2].

Table 2: Age wise distribution of leptospiral IgM antibody positive cases

<table>
<thead>
<tr>
<th>Age group (year)</th>
<th>No. (%) of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upto 15</td>
<td>7 (11.5)</td>
</tr>
<tr>
<td>16-30</td>
<td>24 (39.3)</td>
</tr>
<tr>
<td>31-45</td>
<td>15 (24.6)</td>
</tr>
<tr>
<td>46-60</td>
<td>9 (14.8)</td>
</tr>
<tr>
<td>61-75</td>
<td>6 (9.8)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>61 (100)</strong></td>
</tr>
</tbody>
</table>

4. Discussion

In the last decade, leptospirosis has emerged as a globally important infectious disease\textsuperscript{16}. It is endemic in South East Asia with human infection reported throughout the region\textsuperscript{17}. Major outbreaks were reported in recent years more notably in India, Indonesia, Sri Lanka and Thailand. It is also believed to be endemic in Nepal as ideal conditions exist for transmission of this infection in this country\textsuperscript{18}, however, as with other developing countries, the infection is largely underreported. The non-specific manifestation of the disease, limited laboratory capacity and lack of transversal policy, all contributed to underreporting of cases hence undermining the true prevalence of the disease. For the first time, a significant
number of human cases have been reported by Murdoch et al\textsuperscript{19} in 2004. IgM antibodies appear as early as 3 days after infection and may persist for up to 5 months, although there have been reports of IgM antibodies persisting for years or life\textsuperscript{14}. In the present study, rate of leptospiral IgM antibody positivity was found to be 4.8%. Seroprevalence of leptospirosis differs from one study to other. High rate of seroprevalence has been reported by various authors: 31\% by Deodhar et al from India\textsuperscript{20}, 32\% by Rai et al\textsuperscript{21} from Kathmandu, Nepal, 38.2\% by Morshed et al\textsuperscript{22} from Bangladesh, and 52.7\% by Sharma et al\textsuperscript{23} from Adaman Islands, India. Kandel et al\textsuperscript{8} has also reported Lepotspiral IgM antibody positivity of 37\% from Nepal. However, low seroprevalence of 0.66\% ± 0.34 has been reported by Desvars et al\textsuperscript{16} in global population of Reunion island.

It has been found that the incidence of leptospiral infection is higher in warm climate regions than in temperate regions\textsuperscript{24}. All the leptospiral IgM antibody positive patients in this study were from Terai region of Nepal. Climatic and environmental conditions, host availability, population density and behavioral factors might have favored transmission of the disease in this region.

Occupational activities of the population may influence the seroprevalence of leptospirosis\textsuperscript{16}. Nepal is an agricultural country and about 80\% of the people's occupation is agriculture. Majority (39.3\%) of the seropositive individuals in this study were also agricultural workers. It is believed that the agricultural workers are the most exposed population to leptospirosis\textsuperscript{23}. This could be due to frequent exposure of agricultural worker to rice fields that are often waterlogged, which is the major reason for the survival of leptospires. Domestic animals such as bullocks and buffalo are used for agricultural activities and during harvest season; when the rice is ripe, rodents visit the fields in search of food grain. So the rodents, the bullocks and buffalo and the favorable environment could play a major role in agriculture-based communities for leptospirosis\textsuperscript{16}.

Season wise, highest rate of positivity was seen in autumn season (63.9\%) followed by summer (21.3\%), our findings being in consistent with the report of Deodhar et al\textsuperscript{20} who observed 81\% positivity between the months of July to October, coinciding with the monsoon and post-monsoon season.

No information is available regarding the sex and age group susceptible to leptospiral infection in Nepal. In general, all ages and sexes are susceptible. As adult men work outdoors in high-risk jobs, they are believed to be more vulnerable to infection\textsuperscript{25}. Present study shows high positivity rate in the males of age group of 16-30 followed by 31-45 years, which is similar to the report of Kuriakose et al\textsuperscript{26} who found higher incidence of leptospiral infection in 20-40 years. Males have been shown to have a higher chance of contracting the disease suggesting a strong occupational component in transmission\textsuperscript{17}. In Thailand, despite a decreasing male: female ratio (from a high of 9:1 in 1995 to 3:1 in 2003) male population remained to be more affected than females\textsuperscript{27}. However, there was no difference in male: female prevalence in studies conducted in the Mekong Region in Vietnam\textsuperscript{17,28} where exposure was more related to frequent flooding.

As the mortality rate of the disease has been reported high\textsuperscript{5,29,30}, prevention strategies are of great public health importance. Community people should be educated to increase awareness of the disease and modes of transmission; and environmental control programmes such as rodent control, drainage of flooded areas and occupational hygiene need to be implemented.

The major limitation of this study was inability to use paired sera and to perform other diagnostics in addition to IgM ELISA. A gold standard test such as MAT could not be implemented and sensitivity and specificity of ELISA could not be determined in this study.

5. Conclusion

Leptospirosis remains one of common cause of febrile illness in central Nepal and should be screened. Though serodiagnosis by a microagglutination test (MAT) is the gold standard but is not universally available, IgM ELISA is a reliable serological tool to diagnose leptospirosis. At last, it is recommended that epidemiological surveillance and laboratory capacity across the region need strengthening to determine its true prevalence in the community.

Acknowledgement

We would like to acknowledge all the staffs of clinical laboratory of Chitwan Medical College Teaching Hospital for their help and co-operation to conduct the study.
References