Dengue outbreak in an Indo-Myanmar border area: epidemiological aspects and risk factors

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Received 11 March 2013; received in revised form 2 May 2013; accepted 12 May 2013

Abstract. During October 2007, a large outbreak of suspected dengue fever (DF) was reported in Moreh township, Manipur: the first widespread outbreak in Northeast India. A cross sectional study was carried out in Moreh. The information on outbreak was collected and then described in time, place and person characteristics to arrive at aetiological hypotheses. Two hundred and eighty two serum samples were collected. Ninety one samples were reported positive for acute infection with dengue virus. Co-circulation of all the four dengue virus serotypes (1–4) and concurrent infection of 2 & 3, 1 & 3, and 1 & 4 serotypes was found. Predominant clinical features of the patients were fever (100%), headache (39%), vomiting (9.8%) and joint pain (4.16%). Haemorrhagic manifestation was recorded in one patient who subsequently died. Entomological surveys revealed profuse breeding of Aedes mosquitoes in all the affected municipal wards with high HI (37.5–71.43%), CI (53.84–86.95%) and BI (80–208.33%). Presence of IgM antibody, co-circulation of all the serotypes and concurrent infection with more than one serotype in the same individual confirmed the outbreak due to dengue virus infection. Preventive and control measures undertaken following the epidemiological investigation helped in controlling the outbreak.

INTRODUCTION

Dengue fever (DF) is a rapidly growing public health problem in tropical and sub-tropical countries. Dengue virus belongs to the family Flaviviridae and genus Flavivirus with four antigenically similar but immunologically distinct serotypes, dengue virus (DENV) - 1, 2, 3 and 4 (Halstead, 2000). A total of 2.5 billion people reside in dengue endemic (WHO, 2006) areas and an additional 120 million people travel to the affected areas annually (WTO, 2007). Rapid transportation, industrialization, movement of infected human populations and changing ecology have facilitated its spread to newer areas (Gurugama et al., 2010). In India, 13 states were reported for DF/Dengue Hemorrhagic Fever (DHF) in endemic and epidemic proportions (Govt. of India, Ministry of Health, 2000-2001). In recent years, co-circulation of multiple dengue virus serotypes has been reported with concurrent infections (Gupta et al., 2000; Bharaj et al., 2008). The first case of dual infection with serotypes (DEN-1 and DEN-4) was reported in Puerto Rico in 1982 (Gubler et al., 1985). Dengue fever is transmitted among humans mainly by Aedes aegypti, the urban vector and Aedes albopictus as a maintenance vector in suburban and rural areas (Gratz & Knudsen, 1996). Vector surveillance is an important tool to generate entomological data needed for control strategies and to develop an early warning system (Pant & Self, 1993).

There is no previous report of dengue outbreak in the Northeast (NE) part of India. During the month of November, 2007 a large number of fever cases of unknown origin were reported from Moreh, Manipur. This study included serological, virological and entomological investigation so as to dwell
upon the possible causes of the outbreak and to recommend locally appropriate control measures. Entomological surveys were carried out in human dwellings and peri-domestic areas to identify breeding sites of Aedes mosquitoes.

MATERIALS AND METHODS

Moreh is a small town in Chandel, a hill district of Manipur (24°44' N, 93°58' E). The Indo-Myanmar road is the exact position where Moreh stands today (Figure 1). This border town had a population of 16,737 in 2001. Moreh is divided into 9 municipal wards.

During November 2007, joining hands with the staff and officials of local health authorities of Moreh detection of cases were done by using active as well as passive surveillance. Before we initiated the outbreak investigation, the fever episode was at a declining phase. A total of 11 samples were collected from Regional Institute of Medical Sciences (RIMS) and Shija hospital, Imphal, Manipur. These cases were from 8 different municipal wards of Moreh. We conducted house-to-house survey in all the affected municipal wards. During the active surveillance, by adopting take all approach, a total of 271 serum samples were collected from suspected dengue patients and from persons having history of fever in last 3 months subject to their oral consent. These cases were selected as probable cases of dengue fever as per WHO criteria (WHO, 1997) i.e. acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash or any haemorrhagic manifestations. We plotted an epidemic curve of fever cases, prepared a spot map of dengue cases and calculated ward wise attack rate to describe the outbreak by time, place and person. Informed consent was taken from each patient before collecting blood. This study was approved by an emergent Institutional Ethics Committee, RMRC, ICMR, Assam, India.

Detection of IgM antibodies against dengue virus was done by using MAC ELISA kit obtained from National Institute of Virology, Pune (India). We compared the frequency of exposure variables (Age and sex) through relative risk with 95% confidence interval using EPI INFO version 6.1. Viral RNA was extracted directly from all the serum samples irrespective of their antibody positivity using the QIAamp viral RNA mini kit (Qiagen, Germany). Reverse Transcriptase-PCR was carried out using serotype specific primers for capsid regions of dengue virus (Lanciotti et al., 1992; Harries et al., 1998). The hospitalized patients were also tested for other possible aetiologies like malaria and typhoid. Such infections in these patients were ruled out.

All probable breeding habitats of Aedes mosquitoes were searched in and around the human dwellings of affected wards by systematic sampling searching every 5th house. The immatures present in each container were collected by using spoon/glass pipettes and reared in laboratory till emergence of adults and subsequently species were identified (Rueda, 2004). The computation of ward wise entomological indices, viz. House Index (HI), Container Index (CI) and Breteau index (BI) were carried out as per WHO guidelines (WHO 1999).

RESULTS

Among the 12,813 persons in 8 surveyed wards of Moreh township, 282 clinically suspected dengue fever cases were detected of which 91 (32.27%) were found to be positive. Dengue virus specific IgM antibody was detected in 50 cases and viral RNA in 40 of them. It is noteworthy that only one sample was found to be positive for both IgM antibody and viral RNA. All the hospitalised patients were reported negative for malaria and typhoid. The distribution of fever cases over time gave a step ladder pattern epidemic curve (Figure 2). The ward-wise attack rate of dengue fever cases from the community showed that the area under municipal Ward no. 8 suffered most with an attack rate of 1.29%, followed by Ward nos. 6, 5, 3, 9, 2, 7 and 4 having attack rates 1.16%, 1.03%, 0.96%,
Figure 1. Spot map of dengue cases in the municipal wards showing the gradual spread of the outbreak in Moreh township of Manipur.

Figure 2. Epidemic curve showing the incidence of fever cases during the outbreak in Moreh, Manipur.
0.74%, 0.50%, 0.34% and 0.21% respectively. The spot map (Figure 1) indicated that the first case was reported from ward no.4, subsequently spreading to the adjacent ward nos. 2, 3, 5 and 6 and gradually to all the municipal ward except ward no. 1.

From clinical histories obtained from the persons diagnosed with DF, the predominant complaints were fever (100%), headache (39%), vomiting (9.8%) and joint pain (4.16%). Haemorrhagic manifestation was observed in only one patient.

The majority of patients stated their occupation as farmer (68.4%). The median age of the patients was 33.0±20.0 years. An age wise breakup revealed that 37.4% belonged to the age group of 16-35 years while 28.5% were in the age group 36-55 years. Those less than 15 years of age constituted 23.1% while those in the age group of 55 years and above made up the remaining 11%. Males (Relative risk 1.39, CI=0.82-2.36, P=0.198) and adults (Relative risk 1.58, CI=0.88-2.85, P=0.103) were mostly affected. RT-PCR reveals circulation of all the four dengue serotypes (Figure 3) among which dengue serotype 3 was the most predominant; 26 nos. of serum samples gave an amplicon of the size 290 bp, which is diagnostic of serotype 3. Occurrence of concurrent infection with serotype 1 and 3 in 11 patients, 2 and 3 in 1 patient and, 1 and 4 was observed in 1 patient (Table 1). This is the first report on the prevalence of all the serotypes with concurrent infections from the very first dengue outbreak from NE region of India. One patient was found to be infected exclusively with serotype 1. The patient was a 19 year old female and found positive for dengue IgM antibody. She was admitted to Shija hospital at Moreh on 19th November 2007 with the symptoms of fever, headache and excessive bleeding per vagina for more than 15 days following a miscarriage. Laboratory findings of this patient revealed thrombocytopenia (20,000 cells/Cu mm),
leucocytosis (35000 cells/cu mm) and low haemoglobin level (4.8gm %). In the follow-up investigation, this patient was reported dead.

The noticeable feature of this city is a highly conducive environmental situation for propagation of dengue vectors. There is water scarcity around the Moreh town. Non-availability of municipal water supply enforces the residents to store water in open containers. Water storing habits of the people provide a favourable breeding habitat for *Aedes* mosquitoes. In some houses, people use to grow aquatic flowering plants in large plastic and metal drums in front of their houses which serve as excellent breeding habitats. Dumping of solid waste and used tyres for recycling in many locations are a common sight around the town. These types of manmade water stagnation sources contribute to profuse breeding of *Aedes* mosquitoes in the area.

The indoor and outdoor containers of 64 houses in 8 municipal wards of the township were searched for *Aedes* breeding. HI, CI and BI were calculated (Table 1). The maximum HI (71.43%) was observed in ward no. 8 followed by 7, 2, 6, 9, 5, 4 and 3 with reducing indices respectively. High percentage of CI and BI were also observed in all the wards (Table 1). Among the various types of containers surveyed, household water reservoirs and discarded tyre dumps in the open scrape yards abundant in the township were found to support profuse *Aedes* breeding in the study area. An interesting observation was the co-existence of both *Ae. aegypti* and *Ae. albopictus* in all types of containers in the study area.

**DISCUSSION**

This is the first wide spread outbreak of dengue in NE India documenting co-circulation and high percentage of concurrent infection with two dengue virus serotypes which is a great concern for its probable spread to other adjacent states of the region and also to South East Asian countries. Moreh town is a direct trade centre between India and South East Asian countries. Nagaland, the neighbouring state of Assam also experienced a similar outbreak of dengue in the subsequent year i.e. 2009. The present study identified the breeding habitats of *Aedes* mosquitoes in the area in and around the human dwellings that might have supported the occurrence of the present outbreak.

Epidemic curve with step ladder pattern led us to suspect a mosquito borne disease with an extrinsic and intrinsic incubation period of 10-19 days. Co-circulation of multiple dengue virus serotypes has been reported from many parts of the world including India; however, concurrent infection with more than one serotype of dengue viruses in the same individual is rarely documented. In this outbreak, co-circulation and concurrent infection with two dengue virus serotypes along with the prevalence of both the vectors of this disease viz. *Ae. aegypti* and *Ae. albopictus* have been reported. In recent years, co-circulation of multiple dengue virus serotypes is being increasingly reported with concurrent infection (Bharaj *et al*., 2008; Sarkar *et al*., 2012). But being a first observation of concurrent infection in a very first outbreak from NE India, the high percentage (27.5%) of such infection is noteworthy. Gupta *et al*., (2008) and Bharaj *et al*., (2008) reported concurrent dengue infection in 11.76% and 19% patients respectively. In the present outbreak, predominant combination of DENV-1 and DENV-3 serotypes was observed.

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>No. of patients (%)</th>
</tr>
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<tbody>
<tr>
<td>Single</td>
<td></td>
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<tr>
<td>DENV-1</td>
<td>1</td>
</tr>
<tr>
<td>DENV-3</td>
<td>26</td>
</tr>
<tr>
<td>Multiple</td>
<td></td>
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<tr>
<td>DENV-1 &amp; 3</td>
<td>11</td>
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<tr>
<td>DENV-2 &amp; 3</td>
<td>1</td>
</tr>
<tr>
<td>DENV-1 &amp; 4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
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Predominant combination with these two serotypes has also been reported during other dengue outbreak studies (Bharaj, 2008; Anoop et al., 2010). Concurrent infection with two or more serotypes other than dengue 1 and 3 have been documented in earlier studies (Gupta et al., 2008; Anoop et al., 2010; Pessanha et al., 2011). It has been postulated that concurrent infections by multiple dengue virus serotypes may lead to more severe forms of the disease (Lorono-Pino et al., 1999). Concurrent infections with more than 1 serotype might be expected to occur when multiple serotypes of dengue virus co-circulate in the same population. The feeding behaviour of dengue vector mosquitoes characterized by multiple blood meals in a single gonotrophic cycle (Scott et al., 1997) enables them to become infected by multiple serotypes in each feeding and subsequently transmit the serotypes to a single host.

In the present study, a pregnant woman who had miscarriage and suffered from excessive vaginal bleeding subsequently succumbing to her worsened medical conditions was found to be infected with dengue serotype 1. Thrombocytopenia and leukocytosis observed in the patient was in conformity with other previous studies indicating dengue infection (Lu et al., 2005; Chau et al., 2010). Although the impact of dengue on pregnancy is not fully understood but maternal mortality, vaginal bleeding and miscarriage in pregnant women infected with dengue virus have been reported from different parts of the world (Ismail et al., 2006; Waduge et al., 2006; Adam et al., 2010; Pouliot et al., 2010).

Adult males were more commonly affected in this study which is congruent with North and South Indian dengue outbreaks (Chandralekha & Trikha, 2008; Kumar et al., 2010). Maximum proportion of cases occurred in 16-35 yr age group.

Co-existence of both the vectors in the present dengue outbreak is in conformity with other epidemic studies in different parts of India (Isaacs, 2006; Rajendran et al., 2006). The high indices with maximum attack rate observed in ward no. 8 support the local transmission of the disease. Vector surveillance and containment could go a long way in preventing such outbreaks and checking further spread of the disease in future. The health services should organize awareness camps for preventive measures of dengue; also community participation with emphasis on behavioural changes is essential for sustenance of dengue control. In future, adequate surveillance, co-operation among the community and health services along with study on epidemiological aspects are essential for prevention and eradication of the disease.

The outbreak of DF in Manipur with co-circulation and concurrent infection is the first report from NE region of India. The present study therefore emphasizes the urgent need for undertaking appropriate preventive measures and to improve the surveillance system of the disease/vector so as to check further spread of the disease to other states of the NE region.

Acknowledgements. The financial assistance received from the Indian Council of Medical Research (ICMR), New Delhi, India for conducting this study is gratefully acknowledged. The authors thank Mr. N.K. Baruah, Mr. R. Doloi, Miss Rashmee Topno and Mr. Deep Dutta for their excellent technical assistance received during this study. Guidance provided in course of visit to the affected areas and sample collection by the State Programme Officer (NVBDCP), Manipur and his staff is greatly acknowledged.

REFERENCES


