1. Introduction

Japanese encephalitis (JE) is a leading cause of viral encephalitis in Asia with 30,000–50,000 clinical cases reported annually[1]. As per World Health Organization (WHO) estimates, JE claims 10,000 to 15,000 lives a year[2]. Case fatality rates range from 0.3% to 60%[1]. Epidemics of JE were recognized as early as 1871 in Japan and were common in Japan, Korea, and China in the first half of 1900s. However, with widespread JE vaccinations in Korea, Japan, and Taiwan after 1965, JE cases have been reduced substantially[3]. During the last several decades, JE has spread widely in Asia from originally recognized foci in Japan, Korea, and China. JE was not recognized as a threat in Southeast Asia until 1969, when an epidemic of 685 cases was reported in Chiangmai valley in Thailand[4]. However, the disease subsequently continued to spread throughout Asia and is now endemic in Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Japan, Laos, Malaysia, Myanmar, Nepal, Papua New Guinea, Philippines, Russia, Sri Lanka, Thailand, East Timor, Vietnam, North Korea, South Korea and islands in the Torres Strait of Australia[5].

In India, the disease was first observed in Vellore district in Tamil Nadu in 1955[6]. Since then, the virus is active in many parts of India and outbreaks have been reported from the states of Bihar, Uttar Pradesh, Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Haryana, Kerala, West Bengal, Orissa and union territories of Goa and Pondicherry[7]. Presently, JE is not only endemic in many areas but also spreading to newer areas. In north east (NE) region of India, the disease was first observed in 1976 in Assam and since then the disease has appeared in endemic forms or sporadic outbreaks[8]. Outbreaks of JE have been reported from NE region in Lakhimpur district of Assam between July-August 1989. Later, several outbreaks were reported from Assam in 3 consecutive years from August 2000–2002[10]. Besides Assam, JE cases have been reported from NE states like Manipur and Nagaland[11]. Serological surveys (non human) carried out during 1955 to 1972 showed that JE infection occurred in different parts of Assam and...
Arunachal Pradesh (AP) besides other states[6]. JE cases from two districts of AP viz, Papumpare and East Siang were reported during 2001[11]. The report was based on records of admitted patients who had suffered from meningoencephalitis collected from the diagnosis register maintained in the hospitals without any confirmation of the aetiology. Serological tests were then carried out only on pig sera.

The current study reports the first confirmed JE cases from 10 districts of AP (26°42’N–29°30’N Latitude and 90°36’E–97°30’E Longitude) viz., Changlang, Dibang valley, East Siang, East Kameng, Lohit, Lower Dibang valley, Papumpare, Tirap, West Kameng and West Siang (Figure 1) showing a simultaneous widespread JE invasion in different areas of the state.

2. Materials and methods

In the month of August and September 2005, there was an occurrence of febrile illness in and around Pasighat (150 M above mean sea level) under East Siang District of AP bordering Dhemaji district of Assam situated in the North bank of the river Brahmaputra. Most of the patients admitted to the civil hospital, Pasighat, were presented with symptoms like high fever, headache, vomiting and lost sensoria. An investigation by the RMRC, Dibrugarh was carried out in Pasighat. A total of 30 human blood samples were collected with their written consents. Fifteen porcine sera samples were simultaneously collected from the affected areas to correlate its involvement in the natural cycle of the JE virus. The porcine samples were tested by haemagglutination inhibition (HI) test as described by Clarke et al[12]. JE virus-P20778 strain inactivated antigen was used for the HI test. After the investigations, a prospective study was carried out for 5 years till 2010 to ascertain the presence of JE virus (JEV) among suspected acute encephalitis syndrome (AES) cases and to observe its spread[13]. It was noteworthy that due to lack of advanced medical facilities in the hospitals and Primary Health Centres (PHCs) in the state of Arunachal Pradesh, most of the AES patients were referred to the Assam Medical College & Hospital (AMC&H) and private hospitals located in Dibrugarh, Assam situated across the river Brahmaputra on its southern bank. Some commonly noted symptoms among the patients were fever, headache, vomiting and some neurological disorders. RMRC, Dibrugarh is the designated apex laboratory for JE diagnosis for the NE region of the country. The AES presenting samples were transported from the health care facilities for testing to this centre. The patients’ samples (either sera or CSF or both) were tested for anti-JEV IgM by using IgM MAC– ELISA kits developed by National Institute of Virology (NIV), Pune, India. RNA extraction was done from CSF samples by using QIA amp viral RNA mini kit (Qiagen). The extracted RNA was then used to amplify the JEV specific primers of C-prM region (Forward- 5’ GCAGAAACAAAAACAGAG–3’ and Reverse 5’-ACGGATCTCCTGCTTCGTTG -3’) specifically amplifying at 346 bp[14]. All results obtained were statistically analyzed by Epi info software 6.1.

3. Results

In 2005, in Pasighat area, 12 cases out of 30 human sera samples (40%) were JE IgM positive. Out of the 15 porcine samples collected from this area, 12 (80%) were found to be positive against JE antibodies by HI test. These findings reflected JE virus activity in Pasighat of East Siang district of AP. Besides case positivity from East Siang district, suspected AES cases were recorded from 2006-2010 from 9 new districts of the state. A total of 53 out of 105 AES cases were JE positive (Table 1). The JE reporting districts were Changlang (5.26%), Dibang valley (2.63%), East Kameng (2.63%), East Siang (42.10%), Lohit (18.42%), Lower Dibang

| District          | Year | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 |
|-------------------|------|------|------|------|------|------|------|
| Changlang         | AES  | **JE** | AES | **JE** | AES | **JE** | AES | **JE** | AES | **JE** | AES | **JE** | AES | **JE** | AES | **JE** | AES | **JE** | AES | **JE** | AES | **JE** | AES | **JE** | AES | **JE** |
| Dibang valley     | -    | -    | -    | -    | -    | -    | 2    | 1    | 1    | 1    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| East Kameng       | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| East Siang        | 30   | 12   | -    | -    | -    | -    | 3    | 2    | 1    | 1    | 1    | 1    | 3    | 0    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Lohit             | -    | -    | -    | -    | -    | -    | -    | 10   | 6    | 1    | 1    | 3    | 0    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Lower Dibang valley | -    | -    | -    | -    | -    | -    | -    | -    | -    | 3    | 1    | 1    | 0    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Papumpare         | -    | -    | 1    | 1    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Tirap             | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| West Kameng       | -    | -    | -    | -    | -    | -    | 2    | 2    | -    | -    | -    | -    | -    | -    | 1    | 0    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| West Siang        | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| District information not available | -    | -    | -    | -    | -    | 12   | 12   | -    | -    | -    | 4    | 3    | 12  | 3    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |

*AES: Acute encephalitis syndrome presenting patients; **JE: Japanese encephalitis positive patients.
valley (2.63%), Papumpare (2.63%), Tirap (2.63%), West Kameng (15.78%) and West Siang(5.26%). Residence details of 15 JE positive patients could not be traced. Men sufferers outnumbered women by 0.87 times. Children were at a greater risk compared with adults (Table 2). Out of 19 CSF samples tested by PCR, one was found positive.

4. Discussion

Our findings report the presence of JEV activity in AP from human clinical samples during 2005–2010. During sero–epidemiological study in Pasighat, East Siang district in 2005, high rate of JE positive cases from human clinical samples (40%) and porcine samples (80%) was detected, which identified East Siang as a JE affected district. Apart from the 2005 investigation, subsequently, case positivity was identified in 10 out of 16 districts in the state during 2006–2010. Incidentally, all these 10 districts are also highly endemic for malaria, reporting an annual parasite index (API) of 48.4 and case fatality ratio of 2.66 (National Vector Borne Disease Control Programme, Delhi, India; unpublished data). JE transmission season coincided with that of malaria (May to October). Spread of JE in these hilly and foothill regions of NE India; erstwhile known for malaria endemicity is posing a challenge with regards to proper diagnosis, treatment and management of cases as both JE and cerebral malaria (caused due to Plasmodium falciparum infection) present a somewhat similar clinical picture[15]. A JE vaccination campaign was carried out in the Lohit district during 2010 targeting the ≤15 years’ age group where coverage of 83.09% was recorded. In general, males are reported to suffer more

#### Table 2

| Variables | Disease present | Disease absent | Relative risk | 95% CI | P value |
|-----------|-----------------|----------------|---------------|--------|---------|
| Age       |                 |                |               |        |         |
| ≤15       | 26              | 18             | 1.39          | 0.95–2.05 | 0.09 |
| ≥15       | 25              | 34             | Reference     |        |         |
| Sex       |                 |                |               |        |         |
| Male      | 28              | 31             | 0.87          | 0.60–1.27 | 0.48 |
| Female    | 25              | 21             | Reference     |        |         |

#### Figure 1

Affected districts (●) of AP, India by JE.
than females which were also found in our study[6,16]. As the potential JE vectors of this region, viz., *Culex vishnui* group of mosquitoes are zoophilic and exophilic in nature, they abound near the cattle sheds and pigsties and therefore the folk seem to have a greater exposure to bites by the vector species of mosquitoes[16,17]. JE mainly occurs in children, which was also observed in our study[18].

The study reports confirmed JEV activity in the districts of AP presenting the first serological and molecular evidence of JEV circulation in humans in the state. The ongoing JE vaccination campaigns integrated with vector control measures should be beneficial in deriving a long lasting solution to the problem. Considering the risk factors of transmission of the disease, the primary health care facility needs to be sensitized and improved. Health awareness on JE would go a long way in sensitising and educating the local population and help them initiate vector control as well as organized pig farming and personal protection measures.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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