A serological study of Japanese encephalitis and related flaviviruses in and around Pondicherry, South India

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ABSTRACT
We investigated outbreaks of viral encephalitis that occur periodically in and around Pondicherry (South India) for serological evidence of Japanese encephalitis virus infection. Haemagglutination-inhibition and complement fixation tests or the haemagglutination test alone were done, using Japanese encephalitis, West Nile and Dengue-2 antigens. Japanese encephalitis virus infection was found in 40% of the 111 cases which occurred in the 1983 outbreak. During the 1986 outbreak 33% of the 144 cases showed broad antibody responses to either Dengue-2 or West Nile viruses. Haemagglutination-inhibition antibody profile to Japanese encephalitis, West Nile and Dengue-2 viruses was also present in the normal population. The serodiagnosis of Japanese encephalitis is difficult because of the presence of other flaviviruses in the Pondicherry area.

INTRODUCTION
Virological and serological investigations have confirmed that arboviral infections have been prevalent in India for the last three decades. Reports of Japanese encephalitis have appeared frequently both from the south and the north regions of India; one of the earliest being from the Union Territory of Pondicherry. Since then periodic outbreaks of encephalitis have occurred in and around this area and in the 1980s there has been a spurt in the number of cases admitted to the Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) hospital. The seasonal occurrence seemed to indicate that the outbreaks might be due to the Japanese encephalitis virus. We therefore undertook a serological investigation of outbreaks which occurred in and around Pondicherry in the years 1983 and 1986.

MATERIALS AND METHODS
Sera
A total of 255 patients clinically diagnosed as having encephalitis were investigated for serological evidence of Japanese encephalitis. There were 111 patients in 1983 and 144 in 1986. Paired samples were obtained from only 32 patients in 1983 and 21 in 1986. In the remaining cases single samples were studied because these patients either died or left the hospital against medical advice.

Antigens
Antigens of Japanese encephalitis (JE p 20778), West Nile (WN 22886) and Dengue-2 (DEN-2 p 23055) were provided by the National Institute of Virology, Pune along with corresponding immune ascitic fluids. These were used in the haemagglutination-inhibition tests (HI) and the complement fixation tests (CF).

Both HI and CF tests were done on the serum samples of the 1983 outbreak but only the HI test was done on the 1986 serum samples and the control sera.

HI Test
Control and test sera were titrated according to the method of Clarke and Casals. A proportion of sera of encephalitis cases was also tested by the same procedure after 2 ME treatment to determine the presence of IgM antibodies.

To ascertain the significance of HI antibody distribution obtained with JE, WN and DEN-2 antigens, and to enable interpretation of serological tests among single serum samples from encephalitis cases, the antibody profile of sera from the normal population was investigated. Cut-off titres were then determined for the HI test using standard statistical methods (Mean+2SD).

CF Test
This was performed according to the method of Casals on the 1983 samples. Twenty-one paired sera and 69 single samples of encephalitis cases were tested by this procedure.

RESULTS
A total of 434 patients with encephalitis were admitted to the JIPMER hospital in 1983 (190) and 1986 (244); 89 (46.8%) died in 1983 and 96 (39.3%) in 1986. There
seemed to have been three epidemic peaks in the years 1981, 1983 and 1986 (Fig. 1). The annual and seasonal incidence indicates a regular cyclical occurrence of encephalitis probably due to Japanese encephalitis virus (Fig. 2). The majority of cases occurred during the monsoon season (between the months of September and November) possibly because there is an increase in vector density caused by water pools around paddy fields which form after the rains.

Among the 111 cases studied in 1983, 95 (86%) were below the age of 15 and the majority came from the South Arcot district of Tamil Nadu. Males were more frequently affected than females (2.3:1). In the 1986 outbreak 100 (77%) cases were below 15 and the male to female ratio was 2.1:1.

The common symptoms encountered in 1983 were fever (95%), an altered sensorium (74%), convulsions (51%), headache (36%) and vomiting (19%). The common signs observed were coma (64%), paresis (24%), neck rigidity (23%), hypertonia (21%) and cranial nerve palsies (7%). In 1986, 76% of the patients had fever and 53% an altered sensorium. A few had vomiting, headache, coma, aphasia and neck rigidity.

**HI antibody profile in normal population**

The seropositivity rates for JE antigen among different age groups varied between 50% and 78% while for WN and DEN-2 antigens it was between 50% and 100% and 72% and 95% respectively (Table I). The geometric mean titres of WN and DEN-2 antigens were higher than those obtained with JE antigen in all the age groups. The cut-off titres determined for the HI tests were 80, 160 and 160 for JE, WN and DEN-2 antigens respectively.

**HI and CF antibodies in patients with encephalitis**

Patients were considered to have JE infection if there was a four-fold rise between paired samples and also a four-fold difference between JE and the other two antigens. If only single specimens were obtained, patients were considered to have JE if (i) by the HI test the JE titres were above the cut-off titre and there was a four-fold difference obtained with other antigens and if (ii) by the CF test the titres of the JE antigen were greater than 16 and a four-fold difference was demonstrated with other antigens. If this difference was not demonstrated the 2 ME sensitivity to JE alone (greater than 2 wells) was taken as serological evidence of Japanese encephalitis.

**Table I. HI antibodies to JE, WN and DEN-2 antigens in normal population**

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Sera tested</th>
<th>JE</th>
<th>(%)</th>
<th>GMT</th>
<th>WN</th>
<th>(%)</th>
<th>GMT</th>
<th>DEN-2</th>
<th>(%)</th>
<th>GMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>22</td>
<td>11</td>
<td>(50)</td>
<td>13.7</td>
<td>11</td>
<td>(50)</td>
<td>17.6</td>
<td>16</td>
<td>(73)</td>
<td>29.2</td>
</tr>
<tr>
<td>11-20</td>
<td>15</td>
<td>8</td>
<td>(53)</td>
<td>17.0</td>
<td>14</td>
<td>(93)</td>
<td>49.5</td>
<td>14</td>
<td>(93)</td>
<td>80.0</td>
</tr>
<tr>
<td>21-30</td>
<td>23</td>
<td>18</td>
<td>(78)</td>
<td>37.3</td>
<td>23</td>
<td>(100)</td>
<td>72.1</td>
<td>22</td>
<td>(96)</td>
<td>65.0</td>
</tr>
<tr>
<td>31-40</td>
<td>24</td>
<td>17</td>
<td>(71)</td>
<td>25.2</td>
<td>21</td>
<td>(89)</td>
<td>42.4</td>
<td>23</td>
<td>(96)</td>
<td>44.9</td>
</tr>
<tr>
<td>41-50</td>
<td>21</td>
<td>13</td>
<td>(62)</td>
<td>20.0</td>
<td>20</td>
<td>(95)</td>
<td>39.8</td>
<td>20</td>
<td>(95)</td>
<td>41.3</td>
</tr>
</tbody>
</table>

* A titre of 20 or above was considered positive  GMT geometric mean titres
Using HI and CF tests 44 (40%) of the patients were diagnosed serologically to have had Japanese encephalitis (1983) and in 28 (25%) there was a broad antibody response to the related flaviviruses (Table II).

In the 1986 outbreak only 9 (6%) patients had Japanese encephalitis and 48 (33%) were categorized to be having flavivirus infection using the HI test only.

**DISCUSSION**

There has been an upward trend in the number of patients with encephalitis being admitted to the JIPMER Hospital in Pondicherry. In 1983, age and sex distribution, clinical features, seasonal incidence and mortality all suggested that the epidemic was caused by Japanese encephalitis. Similar observations have been made in South India by other workers.\(^9,10\) However, serologically, in 1983 only 40% of the cases could be categorized to be having Japanese encephalitis and in 1986 only 6% (Table II). The mortality rate in 1986 (39%) was less than that in 1983 (47%).

Casals\(^11\) has demonstrated that exposure to two or more arboviruses of the same antigenic group in succession produces a serum that shows marked cross-reactivity with a variety of viruses in the group. It is possible therefore that the 25% of cases categorized serologically as having flavivirus infection in the 1983 outbreak might have had Japanese encephalitis with prior exposure to West Nile or Dengue type 2 viruses. This assumption is supported by an analysis of the HI antibody profile of the normal population where elevated seropositivity and geometric mean titres were observed in all age groups to WN and DEN-2.
antigens compared to the JE antigen. It therefore appears that, apart from Japanese encephalitis, West Nile and Dengue-2 viruses are also active in this area. Similar observations have been made earlier.\textsuperscript{12}

In the 1983 outbreak there was a significant (p<0.001) difference between the geometric mean titres of acute phase sera and the convalescent phase sera (Fig. 3). This was evident with the JE antigen and not with the others. In 1986 there was only a broad antibody response to WN and DEN-2 antigens and the geometric mean titres between paired samples were not significantly different (Fig. 4).

Prior to 1970, cases of Japanese encephalitis were recorded only from South India. Since then periodic outbreaks have been reported from other parts of India. There was a major outbreak in 1977–8 in Tamil Nadu in the Tirunelveli district in which out of 298 cases, 98 (33\%) died. There was a small outbreak in 1980 followed by a major outbreak in 1981 in which the areas mainly affected were the South Arcot district of Tamil Nadu and Pondicherry (total cases 633, died 152 (24\%)). Japanese encephalitis seems to have established itself in and around this area.

Besides Japanese encephalitis other cross-reacting flaviviruses especially the West Nile and Dengue-2 viruses occur in many parts of India and result in occasional cases of encephalitis. Our findings suggest that the outbreak of encephalitis in and around Pondicherry in 1983 was probably due to the Japanese encephalitis virus and in 1986 due to other related flaviviruses.

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