Japanese Encephalitis and Haemorrhagic Fever with Renal Syndrome Bulletin

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Recent Epidemiological Trend of the Japanese Encephalitis in the Republic of Korea

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The importance of the Japanese encephalitis (JE) from the public health point of view was first recognized during the outbreak in 1949 in Korea when 5616 cases (attack rate 27.8 per 100,000) and 2729 deaths were reported.

Epidemiological data have been available since that date, because the disease was then registered as one of the notifiable communicable diseases of prime importance in Korea.

The initial period which is assumed to be from 1949 to 1958, epidemic JE outbreaks were noticed every three years with over 1000 cases for nine consecutive years. However, the larger scale epidemic in 1958 brought 6897 cases (29.7 per 100,000), and 2177 deaths (9.4 per 100,000) were reported.

The disease persisted with a near epidemic level of 1000 cases annually until 1969. Then the numbers of disease cases dropped precipitously to under 100 cases. The reduced case-level, which is considered to be a recent JE trend, has persisted up to the present, except in 1973 and 1982 when the JE cases slightly increased with the total of 769 and 1197 cases, respectively (Fig. 1).

![Fig. 1. Reported cases of JE in Korea, 1949-1983](image-url)
The intriguing question is how and what factor is responsible for the precipitous drop of JE case level under 100 as was shown in 1969. We would like to relate the above phenomenon of case drop with JE vector, *Culex tritaeniorhynchus*, studies in an endemic area. Jeon-La-Buk-Do province showed abrupt decrease in the year 1969 coinciding with that of JE cases. There might have been other factors involved which remained unknown. One field survey on the JE vectors during the 1970s has shown that the number of *C. tritaeniorhynchus* is increasing in the vicinity of urban areas and case reports in urban areas are increasing more than ever before.

The marked decrease of JE vector population in rural habitats can be attributed to the extensive use of insecticides in rice farming, where the amount of pesticide consumption in recent years has increased (Fig. 2).

It is interesting to point out that Jeon-La-Buk-Do province has been known as the focus of JE epidemics, and the data demonstrate the highest attack rates regardless of epidemic size. In this particular area, further ecological and epidemiological studies have to be made in the future.

In regard to age-groups, JE has been almost entirely a disease of young children: twelve-year data from 1955 to 1966 show that over 90% of the total cases occurred in the age-group of under 14 years. Even in recent low epidemic years, 86.7% of the total cases in 1976 occurred in the young age-group. In the incidence of JE in 1982, the age pattern of patients was slightly changed.

The JE patients were still dominantly children under 14 years of age (72.3%), but there were more patients than in previous years (Fig. 3). The seasonal occurrence of reported cases of JE in 1956-1966 (high epidemic years) and 1972-1980 (low epidemic years) is as follows: of the
total cases reported, 98% were recorded between August and September even though there are different epidemic rates during high epidemic years.

During low epidemic years as shown from 1972 to 1982, 86% of the total cases were found between August and September, and there appeared sporadic cases reported during the month of October as much as 11.7% of the total cases.

The similar seasonal pattern has been observed since 1969, except in 1982, when JE cases were unusually high and the seasonal pattern was similar to those of high epidemic years (Fig. 4).
Japanese Encephalitis in Nepal

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Abstract Japanese encephalitis (JE) was recognized for the first time in Nepal in 1978. A project proposal was developed in 1979 and submitted to His Majesty's Government of Nepal and to WHO/SEARO for approval and technical/financial support, respectively. At the beginning of 1980, an epidemiological surveillance study was started in all endemic and epidemic Tarai districts. Morbidity, mortality serological and entomological study results over seven years have been gathered. A total of 2508 cases in all age-groups (both sexes) were admitted in various hospitals; 886 patients died (case fatality rate: 35.32%). For serological diagnosis 1505 human and 299 animal sera samples were collected: 33.38% of human sera and 33.78% of pig sera were found to have a Japanese encephalitis antibody titre ranging from 1/10 to 1/80 and above. To identify the responsible JE vector, an entomological study team collected 4853 adult mosquitoes and 1216 larval specimen from the endemic/epidemic areas. The percentages of five different genera recorded were Culex: 83.46%, Anopheles: 12.61%, Mansonia: 3.0%, Armiger: 0.76%, and Aedes: 0.21%. Preventive measures such as vector and larvae control, health education, improvement of pig farming were taken, along with special curative measures in hospitals.

INTRODUCTION

Japanese encephalitis (JE) is a zoonotic disease which infects mainly domestic and wild animals and birds; man gets infected occasionally. It occurs over a wide area of eastern Asia from Siberia to India. In Nepal it was first recorded in July 1978 in the western plain Tarai district and, later, affected all 23 Tarai tropical districts. Japanese encephalitis outbreaks were also recorded during that period in the neighbouring districts of Uttar Pradesh and Bihar in India. This arboviral infection is generally maintained in enzootic forms and appears as a focal outbreak under specific ecological conditions.

Culicine mosquitoes have been identified as being the vector of Japanese encephalitis in the Asian continent. But in Nepal, no virus of JE has been isolated from mosquitoes up to the present time.

Pigs and birds (ducks and wild migrating birds) are important amplifying hosts for JE virus, as can be seen from the presence of antibodies in their sera. Infected pigs do not develop any clinical symptoms of illness ex-
cept abortion and still birth but circulate the JE virus in their blood stream so that the mosquitoes get infected and can transmit the virus to man.

The main objectives of the study were to find out the geographical distribution of viral encephalitis incidence, its distribution by age and sex, seasonal variations, mortality and morbidity statistics, case fatality rates by district and, finally, to conclude with remarks for diagnosis of the disease and the control of its incidence.

**MATERIALS AND METHODS**

*Epidemiological study*

Epidemiologically, this disease occurs in Nepal, mainly in areas with tropical and subtropical climate (Tarai districts). Therefore, an epidemiological study was started in 1980 and continued each year until the end of 1984. Teams visited all health institutions of the endemic/epidemic areas. Records of morbidity and mortality for all age and sex groups for the period 1978-1984 were collected in a standard format developed by the Zoonotic Disease Control Section.

*Serological study*

A total of 1505 human blood samples were collected both from active and convalescent cases; other samples were collected from a healthy population in the endemic areas. A team visited all the endemic areas of different zones between March and October of each year.

A total of 299 animal blood samples were collected by the teams fielded by the Zoonotic Disease Control Section, Epidemiology and Statistics Division, Department of Health Services, during the same period each year. About 2-5% of the piglets, ducks and other animals in the endemic areas were randomly covered for blood sample collection. The major endemic areas of Morang, Sunsari, Saptari, Siraha, Bara, Parsa, Nawalparasi, Rupandehi, Kapilvastu, Kailali and Kanchanpur districts were surveyed by the teams.

*Entomological study*

Entomological study teams have visited all endemic areas to collect mosquito vectors responsible for the transmission of the disease to man as well as to other animals. *Culex* Spp. of mosquitoes are the real vectors of this disease. Mosquitoes were collected from pig sheds, animal and human settlement areas during night time, as well as early in the morning, by a standard technique.
Table 1. Cases and deaths of Encephalitis recorded in hospitals in Nepal 1978-1984

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Meechi zone</td>
<td>18-</td>
<td>7</td>
<td>20-</td>
<td>20.00</td>
<td>7</td>
<td>20</td>
<td>20.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Koshi zone</td>
<td>112</td>
<td>69</td>
<td>117</td>
<td>102</td>
<td>63</td>
<td>75</td>
<td>538</td>
<td>29.70</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Sagarmatha zone</td>
<td>7</td>
<td>54</td>
<td>56</td>
<td>5</td>
<td>9</td>
<td>101</td>
<td>317</td>
<td>30.28</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Janakpur zone</td>
<td>39</td>
<td>13</td>
<td>14</td>
<td>65</td>
<td>16</td>
<td>20</td>
<td>187</td>
<td>48.00</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Narayani zone</td>
<td>39</td>
<td>65</td>
<td>158</td>
<td>37</td>
<td>18</td>
<td>317</td>
<td>30.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Bagmati zone</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>91.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Lumbini zone</td>
<td>153</td>
<td>26</td>
<td>217</td>
<td>34</td>
<td>39</td>
<td>496</td>
<td>20.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Rapti zone</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Bheri zone</td>
<td>38</td>
<td>8</td>
<td>53</td>
<td>46</td>
<td>6</td>
<td>150</td>
<td>39.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Seti zone</td>
<td>28</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>532</td>
<td>125</td>
<td>694</td>
<td>41.64</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Mahakali zone</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>15</td>
<td>15</td>
<td>33.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>422</td>
<td>182</td>
<td>622</td>
<td>54</td>
<td>848</td>
<td>243</td>
<td>2,508</td>
<td>35.32%</td>
<td></td>
</tr>
</tbody>
</table>

Note: ( ) Inside the bracket the numbers are death cases.
+ Imported cases
RESULTS

Epidemiological findings

All collected survey data were brought to Kathmandu for compilation, consolidation, tabulation and further analysis. During this seven-year period, over 2508 cases were hospitalized in 10 out of 14 administrative zones (Table 1). Almost all the affected areas are bordered with the Indian States where JE outbreaks were reported in 1978 and also in subsequent years. The highest numbers of cases were admitted and recorded in the Tarai districts of Koshi, Narayani, Limbini and Sati Zones of Eastern, Central, Western and Far Western Development Regions of the country.

On the basis of the epidemiological survey records for 1978 to 1984, an analysis of the hospital records shows that there were very few cases at the end of winter and in early spring, but that the number increased from April to reach a peak in September. Most cases were patients 15 years of age and older or schoolchildren. Less than 15% were children below school age (Table 2). There were more cases in males than in females. Morbidity and mortality record over a seven-year period are also shown in Figure 1. As can be seen from Figure 2, every alternate year is an epidemic year. Seasonal variations are shown on Figures 3 and 4. The total area affected is shown on Figure 5.

Table 2. Cases and death of Encephalitis recorded in hospitals in Nepal by age groups 1978-1984

<table>
<thead>
<tr>
<th>Years</th>
<th>0-4 Years</th>
<th>5-14 Years</th>
<th>15 and above</th>
<th>Age and sex n.s.</th>
<th>Total cases (Death)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>20 (7)</td>
<td>60 (11)</td>
<td>93 (36)</td>
<td>249 (65)</td>
<td>422 (119)</td>
</tr>
<tr>
<td>1979</td>
<td>26 (7)</td>
<td>33 (10)</td>
<td>33 (9)</td>
<td>90 (23)</td>
<td>182 (49)</td>
</tr>
<tr>
<td>1980</td>
<td>78 (25)</td>
<td>231 (73)</td>
<td>293 (125)</td>
<td>20 (8)</td>
<td>622 (231)</td>
</tr>
<tr>
<td>1981</td>
<td>17 (4)</td>
<td>20 (6)</td>
<td>17 (6)</td>
<td></td>
<td>54 (16)</td>
</tr>
<tr>
<td>1982</td>
<td>73 (15)</td>
<td>292 (117)</td>
<td>343 (198)</td>
<td>135 (16)</td>
<td>843 (390)</td>
</tr>
<tr>
<td>1983</td>
<td>25 (4)</td>
<td>116 (16)</td>
<td>102 (16)</td>
<td></td>
<td>243 (36)</td>
</tr>
<tr>
<td>1984</td>
<td>44 (18)</td>
<td>59 (14)</td>
<td>39 (13)</td>
<td></td>
<td>142 (46)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>283 (80)</td>
<td>811 (247)</td>
<td>920 (403)</td>
<td>494 (156)</td>
<td>2508 (886)</td>
</tr>
</tbody>
</table>

% of Total 11.3 (9.02) 32.3 (27.9) 36.7 (45.5) 19.7 (17.6) 100
Fig. 1. Morbidity and mortality of Japanese Encephalitis in Nepal

Fig. 2. Reported cases of JE in Nepal

1949-1984
Fig. 3. Seasonal incidences of Japanese Encephalitis

Fig. 4. Seasonal incidence of Japanese Encephalitis
Serological results

Out of a total of 299 animal samples tested, 101 samples (38.78%) were found to be positive against the JE antigen. Out of a total of 1505 human blood samples collected, 502 (33.36%) had antibodies against JE virus. Serological test results of human and animal sera by district are presented in Table 3.

Table 3. Total human and animal birds serum sample showing HI titre for Japanese Encephalitis from 1980-1984

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Serum samples</th>
<th>Total serum samples showing HI titre for JE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/10</td>
<td>1/20</td>
</tr>
<tr>
<td>1.</td>
<td>Human serum</td>
<td>1505</td>
<td>79</td>
</tr>
<tr>
<td>2.</td>
<td>Animal/Birds</td>
<td>299</td>
<td>38</td>
</tr>
</tbody>
</table>

Entomological results

From 4668 mosquitoes collected, 3875 were specified as Culex with a predominance of Culex tritaeniorhynchus and Culex vishnui complex. During the period 1981 to 1982, vector surveillance activities conducted in the endemic foci of eighteen districts of the Tarai and inner Tarai belt of Nepal have recorded the number of culicine mosquitoes, including the proven efficient JE vector; a breakdown by genus for a total of 2362 female adult mosquito specimens is given in Table 4.

Table 4.1. Percentage of different genus of mosquitoes collected

<table>
<thead>
<tr>
<th>Genus</th>
<th>No. Coll.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Culex</td>
<td>1971</td>
<td>83.46</td>
</tr>
<tr>
<td>(b) Mansonia</td>
<td>70</td>
<td>3.00</td>
</tr>
<tr>
<td>(c) Aedes</td>
<td>5</td>
<td>0.21</td>
</tr>
<tr>
<td>(d) Armigeres</td>
<td>18</td>
<td>0.76</td>
</tr>
<tr>
<td>(e) Anopheles</td>
<td>298</td>
<td>12.61</td>
</tr>
</tbody>
</table>

|       | 2362   | 100.00 |
II. Percentage of different species of Culex collected among the genus Culex

<table>
<thead>
<tr>
<th>No. Coll.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.30</td>
</tr>
<tr>
<td>15</td>
<td>0.76</td>
</tr>
<tr>
<td>10</td>
<td>0.50</td>
</tr>
<tr>
<td>102</td>
<td>5.17</td>
</tr>
<tr>
<td>24</td>
<td>1.20</td>
</tr>
<tr>
<td>122</td>
<td>6.18</td>
</tr>
<tr>
<td>396</td>
<td>20.00</td>
</tr>
<tr>
<td>6</td>
<td>0.30</td>
</tr>
<tr>
<td>1289</td>
<td>65.39</td>
</tr>
<tr>
<td>1971</td>
<td>100.00</td>
</tr>
</tbody>
</table>

DISCUSSION

Japanese encephalitis was a very big threat for people in south western Japan. Through a JE vaccination programme in both humans and pigs, vector control and control of amplifiers, the disease is now under control. It has been observed that JE is distributed widely in eastern and south-eastern Asia and in the Indian subcontinent. From 1978 to 1981 JE was reported in Uttar Pradesh and Bihar States in India, which have a border with Nepal, and also in Madhya Pradesh.

In Nepal, the epidemiological surveillance study was started for the first time at the end of 1980 and in early 1981 in all endemic areas of the country. This was continued every year at the time of the outbreak. On the basis of these studies carried out by different authors in different years, results were published. There were no age and sex limitations in the incidence of the disease. For children below 14 years of age, the mortality rate recorded was 33%, the case fatality rate was 28.7% and the serological incidence rate was 21.88%. Animal sera, particularly from piglets and birds, were also collected from the same communities tested for JE titre, and 33.78% were found positive.

JE is a vector-borne disease which requires not only the vector, but also an amplifier for its spreading. Entomological studies have shown that the main JE vector is the culicine mosquito in Nepal; however, so far, JE virus has not yet been isolated from that mosquito.

Prevention and control of JE could be achieved by three methods, i.e.: vector control, control of amplifiers, and immunization of risk groups of human population.

Attempts have been made to spray malathion liquid (100%) with 50% diesel or kerosene oil in most of the endemic area: it reduced the density of mosquito population for a certain period; larvicides were also used in and around villages and urban areas. So far, no JE vaccination programme has
been started on a government scale covering both people and pigs. Health education campaigns, along with national seminars on JE are conducted regularly.

In the future, virus isolation from animals (particularly from pigs), human cases and vectors (culicine mosquitoes) will be carried out. A pilot epidemiological study in pigs will be carried out just to assess the virus concentration during each month of the year, hence, to show the epidemiological trend. An ecological study of vectors will be carried out. These activities need external financial, as well as technical, assistance.\(^8\)

ACKNOWLEDGMENTS

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REFERENCES


Intense Transmission of Japanese Encephalitis Virus to Pigs in a Region Free of Epidemic Encephalitis

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Abstract: Epidemic Japanese encephalitis recurs annually in the northern provinces of Thailand, but in the southern provinces, cases of human encephalitis are rare. We investigated the transmission of Japanese encephalitis virus (JEV) to pigs in southern Thailand. Blood specimens from one hundred young pigs at abattoirs in three southern provinces were tested for JEV haemagglutination inhibiting (HAI) antibodies. Seventy-four percent were positive. Ten sero-negative sentinel pigs were placed at five locations in one southern province. Seven of the ten pigs developed JEV HAI and JEV IgM ELISA antibodies within two weeks of placement. JEV was isolated from all seven seroconverting sentinel pigs from blood specimens collected 3 to 11 days after placement. Fifteen light-trap mosquito collections at the five locations all included known JEV vectors, some in large numbers. We conclude that there is an intense transmission of JEV to pigs in southern Thailand despite the rate occurrence of human encephalitis in the same region.

Japanese encephalitis virus (JEV), an arthropod-borne flavivirus transmitted by rice-field breeding mosquitoes of the genus Culex, is a cause of major epidemics of encephalitis throughout most of Asia from Japan to
However, the geographic distribution of JEV is not limited to the epidemic region; JEV has also been isolated from mosquitoes in Malaysia, Indonesia, and the Philippines, all countries where epidemic Japanese encephalitis has not been reported. In Thailand, encephalitis epidemics are confined to the northern region of the country where a dramatic increase of hospital admissions for acute encephalitis occurs annually during the months of June, July, and August. No such increase is observed in the south. There are no obvious reasons for this difference.

Pigs are thought to be the main amplifying host of JEV in Thailand. Most adult swines raised in northern Thailand have serum antibodies to JEV, and JEV-seronegative sentinel pigs set out during the epidemic season rapidly seroconvert. However, no published information is available on JEV transmission to pigs in the "encephalitis-silent" southern region. We describe here a study showing that JEV transmission to pigs in southern Thailand is markedly intense.

**MATERIALS AND METHODS**

*Abattoir blood collections*

Abattoirs in the three southern provinces of Choomporn, Surat Thani, and Prachuab Khiri Kan were visited during mid-May 1983. Five-ml jugular or anterior vena caval blood specimens were collected from approximately 30 pigs at each abattoir. An attempt was made to select only young pigs (age 1 to 12 months) from several locations around the province. Pig ages were estimated by animal weight and features. Specimens were transported on wet ice to Bangkok.

*Sentinel pigs*

Young pigs were purchased in Bangkok, bled and kept in screened rooms. The sera were tested for JEV antibodies by the plaque reduction neutralization method, and seronegative animals were selected as sentinels. On 11 July 1983, ten pigs were transported by truck to Choomporn Province for placement. Two pigs were stationed at each of the five locations, all of which were 3 to 10 kilometers apart, in areas near the provincial capital. All sentinels were penned near locally raised pigs. Five ml of venous blood were obtained from each sentinel every two to three days. The serum was immediately separated and stored frozen in dry ice.

*Haemagglutination inhibition (HI) serology*

The sera were absorbed with goose red blood cells, extracted with acetone, and tested for HI activity against 8 haemagglutination units or prototype JEV-infected or Tembusu-infected suckling mouse brain antigens in a microtitre adaptation of the method of Clarke and Casals. Sera producing complete inhibition of haemagglutination at a 1:10 dilution were deemed positive.
Assay for JEV immunoglobulin M (IgM) antibodies

Untreated sera were tested at a dilution of 1:100 in an antibody capture solid phase enzyme linked immunosassay for porcine JEV IgM antibodies.\cite{10}

Virus isolation

The sera which had been obtained during the week before a sentinel pig seroconverted were cultured for JEV in mosquito cell cultures. Initial isolation attempts using C6/36 Aedes albopictus cells\cite{11} were abortive due to toxic effects of the pig sera, even when diluted. Subsequently, serum specimens were diluted 1:4 in L-15 media supplemented with 10% tryptose phosphate broth and 2% foetal calf serum, and 0.25 ml were inoculated onto monolayers of Aedes pseudoscutellaris (LSTM-AP-61) mosquito cells\cite{12} in two-ounce glass bottles. After 30 min. of absorption at room temperature, 5 ml of the same media was added, and the flasks were incubated for 10-14 days at 28°C. Virus growth was detected and identified by indirect immunofluorescent staining with monoclonal antibodies (personal communication, Gould, E.).\cite{13} Isolates were confirmed as JEV by the plaque reduction neutralization method\cite{14} on monolayers of LC-MK2 cells with the antisera in monkeys raised to the prototype Nakayama strain. Triturated mosquito pools were similarly processed by inoculation onto monolayers of C6/36.

Mosquito collections

Light traps (CDC type) were positioned near sentinel pig pens without carbon dioxide bait. Traps were set at dusk and retrieved at dawn. A target sample size of 300 mosquitoes per location was set, when met, collections at that site were discontinued. Specimens were frozen, transported to Bangkok, and sorted according to species. Pools of 10 to 100 mosquitoes of known JEV vector species\cite{15} were processed for virus isolation.

RESULTS

Abattoir serum collections

Seventy-four of 100 abattoir-collected swine sera from the three southern provinces contained JEV HI antibodies (Table 1). The distribution of positive titres was 1:10 in 3; 1:20 in 4; 1:40 in 16; 1:80 in 21; 1:160 in 20; 1:320 in 9; and 1:640 in 1. Titres were two- to eight-fold higher to JEV antigen than to Tembusu virus antigen in 70 of the 74 positive sera. The antibody prevalence in pigs estimated to be older than six months (30/35, 89%) was greater than that among younger pigs (44/65, 68%; by Fisher's Exact Test  p = .04). Although the overall antibody prevalences were not significantly different in the three provinces, the antibody prevalence among
young pigs was higher (p < .05) at Surt Thani than at the other two provinces. Seven sera (five young pigs and two older pigs) contained levels of JEV IgM greater than 100 units, suggestive of infection within the preceding two weeks.

Table 1. JEV HI antibodies in sera from pigs at abattoirs in three provinces in southern Thailand

<table>
<thead>
<tr>
<th>Location</th>
<th>Pig age (est.)</th>
<th>4-6 months</th>
<th>7-12 months</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choomporn</td>
<td>7/13</td>
<td>14/16</td>
<td>21/29</td>
<td></td>
</tr>
<tr>
<td>Surat</td>
<td>18/21</td>
<td>16/19</td>
<td>34/40</td>
<td></td>
</tr>
<tr>
<td>Prachuab</td>
<td>10/31</td>
<td>0/0</td>
<td>19/31</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>44/65</td>
<td>30/35</td>
<td>74/100</td>
<td></td>
</tr>
</tbody>
</table>

Sentinel pigs

Seven of the ten sentinel pigs stationed at five locations in Choomporn province developed JEV HI antibodies and high levels of JEV IgM antibodies (400 units) during the 4 weeks of monitoring (Table 2).

Table 2. JEV infections in sentinel pigs in Choomporn Province

<table>
<thead>
<tr>
<th>Pig</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1-1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A1-2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A1-3</td>
<td>0</td>
<td>4*</td>
</tr>
<tr>
<td>A2-1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A2-2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A2-3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B1-1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B1-2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B2-1</td>
<td>0*</td>
<td>0</td>
</tr>
<tr>
<td>B2-2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers shown are JEV HI titres of sentinel pig sera. Pigs were set out in the afternoon of 11 July 1983.

*Asterisks denote specimens from which JE virus was isolated.
All seven animals seroconverted within 15 days of placement. At three locations both pigs became infected; and one location one did and at another location, neither did. JEV was isolated from all seven seroconverting pigs from blood specimens drawn on days 3, 5, 7, 8, 9, 10 and 11 after placement, respectively.

Mosquito collections

Fifteen light-trap collections were made. Trap yields varied from 19 to 2871, with a mode of 129 mosquitoes (Table 3). Thirty-seven species were identified. Known JEV vector species were found in all fifteen collections. *Culex tritaeniorhynchus* accounted for 46% of all specimens; 10 or more *C. tritaeniorhynchus* were present in 12 of the 15 traps. A single collection at location B1, where both sentinel pigs developed JEV viremia in 5 days or less, contained the greatest number of *C. tritaeniorhynchus* (1447 mosquitoes); the lowest mean trap yields of *C. tritaeniorhynchus* 924 and 20 mosquitoes per night) occurred at locations A1 and B1 where two pigs and one pig, respectively, remained uninfected. JEV was not isolated from any of the 2792 mosquitoes of known vector species.

Table 3. Mosquito light-trap collections in Choomporn Province

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>Total number collected</th>
<th>CT*</th>
<th>CG*</th>
<th>CV*</th>
<th>CF*</th>
<th>Other*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>12 Jul</td>
<td>19</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>14 Jul</td>
<td>80</td>
<td>12</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>20 Jul</td>
<td>121</td>
<td>39</td>
<td>20</td>
<td>4</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>21 Jul</td>
<td>134</td>
<td>42</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>82</td>
</tr>
<tr>
<td>A2</td>
<td>12 Jul</td>
<td>25</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>16 Jul</td>
<td>55</td>
<td>02</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>22 Jul</td>
<td>232</td>
<td>138</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>81</td>
</tr>
<tr>
<td>A3</td>
<td>14 Jul</td>
<td>96</td>
<td>26</td>
<td>20</td>
<td>4</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>16 Jul</td>
<td>139</td>
<td>59</td>
<td>17</td>
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<td>1</td>
<td>62</td>
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<tr>
<td></td>
<td>24 Jul</td>
<td>129</td>
<td>73</td>
<td>6</td>
<td>9</td>
<td>1</td>
<td>40</td>
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<tr>
<td></td>
<td>2 Aug</td>
<td>378</td>
<td>250</td>
<td>106</td>
<td>0</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>B1</td>
<td>13 Jul</td>
<td>41</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>15 Jul</td>
<td>244</td>
<td>30</td>
<td>32</td>
<td>11</td>
<td>27</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>18 Jul</td>
<td>219</td>
<td>31</td>
<td>31</td>
<td>1</td>
<td>3</td>
<td>153</td>
</tr>
<tr>
<td>B2</td>
<td>13 Jul</td>
<td>2871</td>
<td>1447</td>
<td>45</td>
<td>132</td>
<td>61</td>
<td>1186</td>
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<td></td>
<td>Totals</td>
<td>4753</td>
<td>2175</td>
<td>325</td>
<td>168</td>
<td>124</td>
<td>1961</td>
</tr>
</tbody>
</table>

*CT = C. tritaeniorhynchus; CG = C. gelidus; CV = C. vishnui group; CF = C. fuscocephalus; Other = 33 other species.
Human encephalitis in Choomporn Province

The human population of Choomporn Province in 1980 was 330,000. From 1974 through 1983 an average of two cases of encephalitis per year in Choomporn were reported to the Royal Thai Government Ministry of Public Health. During 1983 only one case of unspecified viral encephalitis was reported from Choomporn Province (attack rate 0.3 per 100,000), while for the year 1983 a total of 1179 cases were reported from the 17 northern Thai provinces (overall attack rate 12.2 per 100,000; Sujart Jutanesen, personal communication).

DISCUSSION

Epidemic Japanese encephalitis recurs every year during the early rainy season in northern Thailand. Epidemics are abrupt and dramatic, quickly filling provincial hospital pediatric wards. Seasonal attack rates often exceed 100 per 100,000 children under the age of 15 years. Epidemic Japanese encephalitis also occurs in northeastern and central Thailand with lower attack rates than in the north. In contrast, epidemic Japanese encephalitis has never been reported in the southern region. Sporadic cases of encephalitis are reported from the southern provinces, but the total yearly attack rates are only one tenth to one fifth of those in the north, and there is no seasonal peak. Serologic evidence of JEV infection has been obtained in a few cases from the southern provinces, but there has never been a systematic study in the region because encephalitis has not been perceived as a significant problem. The epidemiology of human Japanese encephalitis in southern Thailand appears similar to that in Malaysia and the Philippines, where infrequent sporadic cases of Japanese encephalitis have been documented but where epidemic encephalitis is unknown. The geographic zone of distribution of JEV extends well beyond the epidemic regions in Asia and South-East Asia, for JEV has been isolated from mosquitoes in Java, Luzon, and Sarawak, and JEV neutralizing antibodies have been detected in a high proportion of animal and human sera in these tropical Asian regions.

Given the complex natural transmission cycle of JEV, any attempt to identify the critical missing factor(s) in these "encephalitis-silent" areas is a formidable task. Nonetheless, information gleaned in a study of JEV transmission in a silent area might lead to the application of more rational control efforts in the epidemic regions. There are five key elements to be considered in an analysis of transmission of a vector-borne zoonosis: (1) viruses, (2) vectors, (3) vertebrate hosts, (4) climate and (5) humans. None of the known differences between northern and southern Thailand regarding any of these elements can fully account for the different patterns of JEV transmission. Human population, rice fields, and pig densities are comparable, and annual rainfall and the temperature patterns in the upper isthmus provinces of the south are quite similar to some of the severely affected provinces in the north.
As a first step, we sampled pigs at abattoirs for serologic evidence of previous JEV infection. In the north, JEV transmission to pigs is lowest during January through March, and remains low until June. We sampled during May, and found that 68% of pigs born in the south within the preceding six months had already developed serum JEV HI antibodies. Eight percent of all young pigs have high serum levels of JEV IgM, consistent with JEV infection within the preceding two weeks. We then selected the province of Choomporn for more detailed study. The rate of seroconversion of sentinel pigs in Choomporn, 7 of 10 within two weeks, was actually greater than the rate we had observed during the peak of the previous epidemic season in the northern province of Kampangphet, in which 9 of 17 pigs seroconverted within three weeks. In one of the sentinel pigs in Choomporn, viremia occurred within three days; transmission to this animal must have occurred within hours of placement. Virus strains recovered from all seven animals were clearly identified as JEV both by plaque reduction neutralization with polyvalent antisera and by immunofluorescent staining of infected cells with monoclonal antibodies. Despite this evidence of intense transmission of JEV to pigs, only one case of human encephalitis was reported from Choomporn Province. Other cases may have occurred and gone unreported, but certainly no epidemic occurred.

One possible explanation for our findings is that the southern Thai JE strains are avirulent for man. Significant variations in virulence among strains of St. Louis encephalitis virus, West Nile virus and JEV have previously been documented in animal challenge studies. Laboratory studies are currently underway to compare the Choomporn JEV isolates with strains collected in northern Thailand. Another possible explanation is that the mosquitoes which transmit JEV to pigs in the south are unable to transmit the virus to man. Although mosquito light trap collections in Choomporn included ample numbers of species known to transmit JEV, it is possible that the vector competence of the strains of these species in southern Thailand is inferior to that of northern strains of the same species. Lastly, the immune status of the Choomporn human population with respect to dengue may be important. Studies of the prevalence of dengue and JEV neutralizing antibodies in humans in southern Thailand should be conducted.

REFERENCES


JEV in Pigs


IgM Capture ELISA for Serodiagnosis on Japanese Encephalitis and its Differentiation from Dengue Virus Infection

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Abstract Antibody-capture ELISA was used to measure IgM-class of antibodies against Japanese encephalitis (JE) and dengue viruses in sera from JE patients in Japan, or encephalitis and dengue haemorrhagic fever (DHF) patients in Thailand. Examination on titre distribution and the ratio of the titres against JE and dengue antigens led to the diagnostic criteria on JE and dengue virus infections. The criteria were used in the serodiagnosis on DHF cases in Burma showing the effectiveness for early diagnosis. The method was useful to differentiate JE and dengue virus infections in the areas where multiple flavivirus infections are prevailing.

INTRODUCTION

Although the number of human JE cases in Japan decreased precipitously after the epidemic in 1966 and the annual number of cases has been kept under 100 since 1972 (Statistics of the Ministry of Health and Welfare of Japan), there have been many outbreaks in other parts of the East, South-East to South Asia constituting a grave public health problem. On the other hand, DHF has been one of the most serious virus infections affecting a number of children in South-East Asia since its first epidemic in 1953. 17, 18, 30
In the case of Thailand, many encephalitis cases have been reported from the Northern area, around Chiang Mai, since its first outbreak into an epidemic in 1969.\textsuperscript{16, 30} Serodiagnosis on JE and DHF has been carried out mostly by the haemagglutination inhibition (HI) or complement fixation (CF) tests.\textsuperscript{11} Diagnostic criteria on JE by the HI or CF tests have been worked out and efficiently used in routine serodiagnosis because JE virus is essentially the only flavivirus prevailing.\textsuperscript{24} Although the HI test is simple, it could not sometimes distinguish JE and dengue viruses belong to the same family of Flaviviridae. As they possess common antigens, cross reactions occur quite often, especially in secondarily infected cases in areas were multiple flaviviruses including JE and dengue are prevalent, like in Thailand. IgM-class antibodies having been shown to be more specific than total immunoglobulin antibodies against flaviviruses,\textsuperscript{27, 29} separation of IgM antibodies by sucrose gradient sedimentation followed by HI was used in the differential diagnosis of JE and dengue in Northern Thailand.\textsuperscript{13, 14} Burke and his co-workers utilized radioimmunoassay or ELISA in the serodiagnosis of JE in Thailand with remarkable success.\textsuperscript{8, 9, 10} We have been studying the application of the ELISA to the serodiagnosis and seroepidemiological survey of JE virus infections in human, swine and bovine populations.\textsuperscript{1, 6} Recently, we applied the IgM-capture-ELISA to the differential diagnosis of JE and dengue virus infections in South-East Asia, and the results are presented in this paper.

**MATERIALS AND METHODS**

*Serum specimens*

The following paired sera were used in the tests: 33 DHF and 19 encephalitis patients in Chiang Mai, Thailand.\textsuperscript{12} 11 DHF in Chanthaburi, Thailand,\textsuperscript{15} 42 JE cases in Nagasaki, Japan, 168 DHF cases in Rangoon, Burma. Single sera from 149 individuals in Hokkaido (JE non-endemic area) were kindly supplied by Dr K. Ishii in Hokkaido University School of Medicine and were used as negative controls. Sera from Thailand and Burma were classified either into primary, secondary, presumptive secondary, or not dengue cases, using the results of the HI tests according to the WHO guideline.\textsuperscript{51} All the 42 JE cases in Japan were serodiagnosed by the criteria of National Institute of Health of Japan.\textsuperscript{24}

*ELISA antigens*

Each of the four types of dengue viruses, i.e. type-1 Hawaiian (D1), type-2 New Guinea B (D2), type-3 H87 (D3), and type-4 H241 (D4), was inoculated to *Aedes albopictus* clone C6/36 cell.\textsuperscript{19} The infected culture fluid was harvested after 7 days of incubation of 28°C and was used as dengue ELISA antigen without further purification. Formalin-inactivated and purified JE (Nakayama strain) vaccine concentrate\textsuperscript{26} was kindly
supplied by the Research Foundation for Microbial Diseases of Osaka University and used as JE ELISA antigen. The same results were obtained using JE virus infected C6/36 cell culture fluid or sucrose-acetone extracted antigen from infected suckling mouse brains.

**Antiflavivirus IgG**

High titered DHF patients sera from Thailand or Burma were pooled and dialysed against 0.005 M sodium phosphate buffer, pH 8.0, at 4°C overnight, and applied on DEAE Sephadec column equilibrated with the same buffer. The IgG that was not bound to the column was eluted as the first peak of $OD_{280}$ and was concentrated by negative pressure dialysis.

**Preparation of enzyme conjugate**

Concentrated anti-flavivirus human IgG was labeled with horseradish peroxidase according to Nakane and Kawaoi.23

**ELISA**

The IgM-capture method was somewhat modified from that of Duremeyer et al.12. The 96-well microplate was coated with anti-human IgM (u-chain specific) goat IgG diluted in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) at 37°C for 1 hour. The wells on the plate were emptied and washed with PBS-Tween 3 times for 3 minutes each. Test sera and negative serum diluted 1:100 and serial two-fold dilution starting from 1:100 dilution of the standard positive serum with predetermined endpoint titre were distributed in each separate well and the plate was incubated at 37°C for 1 hour. The plate was emptied and washed as above. Virus antigen was distributed and incubated again at 37°C for 1 hour. The plate was washed as above and reacted with anti-flavivirus IgG conjugated with peroxidase. After one hour’s incubation at 37°C, the plate was emptied and washed, followed by the peroxidase reaction using o-phenylene diamine and hydrogen peroxide as substrate. The reaction was stopped by sulfuric acid and color intensity was measured by the Micro ELISA Autoreader to record $OD_{490}$ using $OD_{650}$ as reference wavelength. The titre of each test serum was estimated by comparing the color of each specimen with those developed by serial dilution of the standard positive serum.20,22

**Detection and removing of rheumatoid factor (RF)**

The RF was detected and removed by the latex agglutination method.
RESULTS

Frequency distribution of IgM-ELISA against JE and dengue antigens in JE patients in Japan, and encephalitis and DHF patients in Thailand

As shown in Table 1, all but one negative, the sera from 42 JE cases in Japan and all the 4 primary encephalitis cases in Thailand showed that their IgM-ELISA titres against JE antigen were over 200, while their IgM-ELISA titres against dengue antigens were under 400.

Table 1. IgM-ELISA titre distribution of healthy people in Hokkaido and JE patients in Japan, encephalitis and DHF patients in Thailand, measured by JE and each of the 4 types of dengue antigen.

<table>
<thead>
<tr>
<th>Serum specimen</th>
<th>Assay antigen</th>
<th>99</th>
<th>199</th>
<th>399</th>
<th>799</th>
<th>1599</th>
<th>3199</th>
<th>6399</th>
<th>12799</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy people</td>
<td>JE 144</td>
<td>5</td>
<td>199</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>in D1</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hokkaido</td>
<td>D3 140</td>
<td>9</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>D4 137</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>JE patients</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>D1 41</td>
<td>1</td>
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<td>Primary JHE</td>
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<td>3</td>
<td>1</td>
<td>2</td>
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<tr>
<td>encephalitis</td>
<td>D1 8</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>in D2 4</td>
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<tr>
<td>D3 5</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Thailand</td>
<td>D4 1</td>
<td>4</td>
<td>3</td>
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<td>Secondary JHE</td>
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</tr>
<tr>
<td>in D2 6</td>
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<td>2</td>
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<td>D3 7</td>
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<td></td>
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</tr>
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<td>Thailand</td>
<td>D4 2</td>
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<td>1</td>
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<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2 2</td>
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<td>1</td>
<td></td>
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<td>1</td>
</tr>
<tr>
<td>in D1 11</td>
<td>15</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2 4</td>
<td>11</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td>D3 13</td>
<td>13</td>
<td>8</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D4 7</td>
<td>19</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures represent number of individuals.
Six out of 11 secondary encephalitis cases in Thailand showed that their IgM-ELISA titres against JE were over 200. However, one of the secondary encephalitis in Thailand showed more than 400 IgM-ELISA titre against dengue type-2 antigen and its titre against JE was low. The result suggests that the cases could be dengue encephalopathy rather than JE. All the other encephalitis cases showed IgM titres which were less than 400 against all dengue antigens.

The IgM titres of primary DHF cases were over 400 against dengue type-1 and type-2, and over 200 against type-3 and type-4 dengue antigens, but their titres were under 200 against JE antigen. The IgM titres of 40 secondary DHF cases in Thailand scattered in broad range, with 30 cases (75%) showing IgM titres which were over 200 against at least one of the four types of dengue antigens. All the sera from Hokkaido, except a single serum with IgM titre over 200 against dengue type-4 antigen, showed IgM titre under 200 against all the antigens tested.

**Comparison of the IgM-ELISA titre against JE and dengue antigens in each test serum.**

Table 2 shows the number of test sera showing various titre ratios against JE and dengue antigens. All but one (negative case) JE patients in Japan, 7 out of 8 primary and 4 out of 11 secondary encephalitis in Chiang Mai showed JE IgM-ELISA titres which were four-fold or more higher than the titres against dengue antigens. The remaining one primary encephalitis in Chiang Mai showed similar results except against D4 antigen. In the case of two secondary encephalitis in Chiang Mai, the IgM-ELISA titre against JE was under 100, with over 200 IgM titre against at least one of the four types of dengue antigens. These cases were more likely dengue encephalopathy rather than JE.

In the case of all the 4 primary and 22 out of 40 (55%) secondary DHF, their IgM titres against at least one type of dengue antigen were four-fold or more higher than those against JE antigen. In the case of two primary DHF, their IgM titres against D1 and D2 were four-fold or more higher than D3 and D4 antigens. In three of the 22 secondary DHF, IgM titres against 11 types of dengue antigen was four-fold or more higher than the titres against other types of dengue antigens.

RF was detected in one of the Hokkaido sera with IgM titre over 200 against D4 antigen, which, however, decreased to less than 100 after removal of RF. In seven out of the 42 JE patients in Japan, RF were detected. However, their titre did not change significantly after removal of RF. RF were not detected in sera from Thailand.

From the results described above, we propose the following diagnostic criteria for JE and dengue infections by IgM-ELISA. The case can be considered as JE when its IgM-ELISA titre against JE was over 200 and four-fold or more higher than against any of the four types of antigens. On the other hand, the case can be considered as dengue infection when its IgM-
Table 2. Ratio of IgM-ELISA titres against JE and dengue antigens in sera from JE patients in Japan, encephalitis and DHF patients in Thailand.

<table>
<thead>
<tr>
<th>Ratio of IgM-ELISA</th>
<th>JE in Japan</th>
<th>Encephalitis</th>
<th>DHF in Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Secondary</td>
<td>Primary</td>
</tr>
<tr>
<td>JE/D1 ≥ 4</td>
<td>41</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>JE/D2 ≥ 4</td>
<td>41</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>JE/D3 ≥ 4</td>
<td>41</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>JE/D4 ≥ 4</td>
<td>41</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>D1/JE ≥ 4</td>
<td>2</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>D2/JE ≥ 4</td>
<td>2</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>D3/JE ≥ 4</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>D4/JE ≥ 4</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>11</td>
<td>4</td>
</tr>
</tbody>
</table>

ELISA titre against dengue antigen(s) was over 200 and four-fold or more higher than against JE antigen. Based on these criteria, 41 out of 42 (97.6%) JE patients in Japan, 7 out of 8 (87.5%) primary and 4 out of 11 (36.4%) secondary encephalitis in Chiang Mai were diagnosed as JE. On the other hand, all the 4 (100%) primary and 22 out of 40 (55%) secondary DHF cases and 2 out of 11 (18.2%) secondary encephalitis in Thailand were dengue infections.

Serodiagnosis on DHF in Burma

The same diagnostic criteria were applied to 168 pairs of DHF sera obtained from Rangoon, Burma, and the results are summarized in Table 3. Fifteen out of 18 primary, all the 24 secondary, and all the 60 presumptive secondary cases were diagnosed as dengue infections by IgM-capture ELISA. Moreover, 39 out of 66 not dengue cases by HI also possessed diagnostic levels of IgM-ELISA titres against dengue antigens. All the DHF specimens did not show significant levels of IgM-ELISA titres against JE antigen. Table 4 shows percent positives of serodiagnosis on DHF by IgM-ELISA using different types of dengue antigens and acute (S1), convalescent (S2), or both sera. The results show that D4 antigen gave the highest positives, 60.5% with S1, 61.7% with S2, and 76% when both S1 and S2 were used.
Table 3. Comparison of the serodiagnosis by HI and IgM-ELISA on DHF patients in Tangoon, BURMA.

<table>
<thead>
<tr>
<th>Serodiagnosis by HI</th>
<th>Total number of patients</th>
<th>Positive by IgM-ELISA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>18</td>
<td>15 (83)</td>
</tr>
<tr>
<td>Secondary</td>
<td>24</td>
<td>24 (100)</td>
</tr>
<tr>
<td>Presumptive secondary</td>
<td>60</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Not dengue</td>
<td>66</td>
<td>39 (59)</td>
</tr>
<tr>
<td>Total</td>
<td>168</td>
<td>138 (82.1)</td>
</tr>
</tbody>
</table>

DISCUSSION

Burke and Nisalak (1982) reported the diagnosis on JE by IgM-capture radioimmunoassay and later the assay was extended to use ELISA. In their method, they introduced 20% normal human serum in antigen diluent, probably in order to block direct binding to the catching antibody of trace IgM which could remain in the IgG fraction prepared from DHF patients’ sera and conjugated with peroxidase, causing high background color reaction. In our test, we carefully removed IgM from human IgG by column chromatography on DEAE Sephacel, and addition of normal human serum could be omitted. This is practically important, since such normal human sera are rather hard to obtain in Southeast Asia because of the high endemicity of flavivirus infections. Also the method to estimate the “titre” of test sera by comparing the color intensity with those by the serial dilution of the standard positive serum gave better reproducibility than by the P/N ratio which Burke and his group have been using.

DHF has been a serious clinical and public health problem in many South-East Asian countries and a better method of serodiagnosis was required, especially to differentiate dengue from other flavivirus infections like JE, which may coexist with dengue virus infections in the same geographic areas. The results in this report showed that IgM-ELISA could give fairly good answer to this problem, because of its simplicity, rapidity and capacity to get the results even with single serum specimen, and differential diagnosis on dengue and JE.

REFERENCES


Immunogenicity of JE Nakayama and Beijing-1 Vaccines

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*Department of Virology and Rickettsiology, National Institute of Health
**Tokyo University School of Medicine

Abstract The immunogenicity of Japanese encephalitis current vaccine (Nakayama strain) and trial manufactured vaccine (Beijing-1 strain) were compared by neutralization test (NT). In any vaccine, NT titre against the homologous strain was higher than that against heterologous strain. NT titre against Jaga-01 strain was intermediate between those two. The regression coefficient of NT titre of Nakayama vaccine against Nakayama strain and Beijing strain was 0.67 and rather specific to Nakayama strain. That of Beijing-1 vaccine against Beijing strain and Nakayama or Jaga-01 strain was 1.07 and 1.16, respectively. These results suggest that Beijing-1 vaccine has a wide immunological spectrum.

Japanese encephalitis (JE) cases have drastically decreased in Japan in the past fourteen years and the number of cases are now less than 100 per year. However, in East- and South-East Asia, including the Republic of Korea, China, Thailand, Nepal and India, JE is prevailing and has become an important disease.

The current commercially available vaccine in Japan is manufactured with Nakayama-NIH strain. However, among JE strains isolated in these areas, including Japan, there are some antigenic differences; they are divided grossly into three antigenic groups, Nakayama-NIH, Jaga-01 and their intermediate types by absorption haemagglutination inhibition test (HI)*, absorption neutralization test (NT)* or HI using monoclonal antibodies. So the efficacy of Nakayama vaccine has been argued in the epidemic area where the antigenically different strains are prevailing.

In 1984, a trial vaccine was produced by the Research Foundation for Microbial Diseases, of Osaka University, using Beijing-1 strain which has the Jaga-01 type antigenicity. We compared the immunogenicity of Beijing-1 vaccine with Nakayama vaccine, and it was suggested that Beijing-1 vaccine might be superior to Nakayama vaccine because of a wider antigenic spectrum.

The Nakayama vaccines used the reference vaccine for the National
Quality Control and commercially available ones; the Beijing vaccines were the interim reference and trial ones manufactured by the vaccine producers. Suckling mice brains infected with JEV strains were homogenized with phosphate buffered saline (PBS) containing 10% calf serum (CS) in 10% or 20% suspension, then were centrifuged at 8000 x g for 30 min. and stored at 80°C.

Four-week old ddY female mice were immunized two times with 0.5 ml of diluted vaccine intraperitoneally at a one-week interval. Vaccine dilution was performed with PBS to appropriate dilution (1:4 to 1:32, in most cases 1:32). One week after the second immunization, blood was taken from each mouse, and 11 individual sera were collected per dilution of vaccine or lot tested. The pooled sera were diluted 10 times with Hanks’ BSS containing 5% CS (Hanks’) and were inactivated at 56°C for 30 min. Chick embryonic fibroblast cells were prepared from 9-day old embryonated eggs in lactalbumin-Earle’s medium containing 5% CS with a cell density of 4 x 10^6 cells/ml per petri dish, 7 cm in diameter, and were incubated in a CO_2 incubator overnight. The next day, equal volumes of test sera and virus suspension diluted with Hanks’ were mixed and incubated at 37°C for 90 min. for neutralization. For virus inoculation, 0.4 ml of the mixture was used in each petri dish and absorbed at 37°C for 90 min. After the absorption, 8 ml of first overlay medium was added in each petri dish. After another two days, the second overlay was performed. Plaques were counted on the 5th and 6th days.

NT titre was calculated from the correlation chart between serum dilution and plaque reduction rate and expressed as log_{10} for serum dilution to reduce 50% of plaques in the control dish. 4

Table 1. Average NT titres of Beijing-1, Nakayama and P3 vaccine against three virus strains.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>No. examined</th>
<th>Nakayama</th>
<th>NT titre against Beijing-1</th>
<th>JaGAr-01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beijing-1</td>
<td>6</td>
<td>1.51</td>
<td>2.31</td>
<td>1.86</td>
</tr>
<tr>
<td>Beijing-1</td>
<td>12</td>
<td>1.85</td>
<td>2.32</td>
<td></td>
</tr>
<tr>
<td>Nakayama</td>
<td>13</td>
<td>2.61</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>Nakayama</td>
<td>2</td>
<td>2.31</td>
<td>1.55</td>
<td>1.78</td>
</tr>
<tr>
<td>P3</td>
<td>11</td>
<td>2.17</td>
<td>1.55</td>
<td></td>
</tr>
</tbody>
</table>

Titre is expressed as log_{10}

Table 1 shows the average NT titre of the two kinds of vaccines. Nakayama and Beijing-1 against three virus strains, Nakayama-NIH, Beijing-1
Immunogenicity of JE Nakayama and Beijing-1 Vaccines

and JaGAr-01. It is reasonable that the NT titre of one vaccine against the homologous strain was higher than that against the heterologous one. The NT titre against JaGAr-01 was intermediate between them.

The regression coefficients of both vaccines against the homologous and the heterologous strains were examined.

Figure 1 shows the regression lines of two vaccines against Beijing-1 and Nakayama strains. In Beijing-1 vaccine, the regression coefficient is nearly 1; in contrast, that of Nakayama vaccine is 0.67. This indicates that Nakayama vaccine produces a rather specific NT antibody to Nakayama strain and NT-related epitope(s) in Nakayama strain may be partly defective for Beijing-1 strain. On the contrary, it is supposed that the difference of the NT titre of Beijing-1 vaccine against Beijing-1 and Nakayama strains may not be qualitative but quantitative because the regression coefficient is nearly 1 and Beijing-1 vaccine may produce full sets of NT antibodies against epitopes of both Beijing-1 and Nakayama strains. These results, were confirmed from the data collected from seven vaccine producers' own assay documents (Fig. 2).

![Figure 1. Regression of NT titre of B or N vaccine against Beijing and Nakayama strains. Numerals of axis show NT titre against indicated strain and expressed as log_{10}.](image-url)
For Beijing-1 vaccine, NT against JaGAr-01 strain was also compared. Again the regression coefficient became roughly 1 (Fig. 3).

Fig. 3. Regression line of NT titre of B vaccine against Beijing and JaGAr-01 strains.
Immunogenicity of JE Nakayama and Beijing-1 Vaccines

Fig. 4. Regression line of NT titre of Chinese vaccine against Beijing and Nakayama strains.

We also examined the Chinese vaccine manufactured from the primary hamster kidney cell grown P3 strain, following a request from Dr. Li Homin, National Institute for the Control of Pharmaceutical and Biological Products, China (Table 1 and Fig. 1). This vaccine shows a higher NT titre against Nakayama strain than against Beijing-1 strain. However, the regression coefficient of 0.93 indicates that this vaccine is rather a Beijing-1 type vaccine in its immunogenicity.

From these results, it is suggested that Beijing-1 vaccine has a wide immunological spectrum and will cover Nakayama type epidemics as well as JaGAr-01 type epidemics though NT titre against the heterologous strain is lower than against the homologous strain. Work is in progress against Mie 44-1 strain, the intermediate type of JEV.

REFERENCES


The Role of Peritoneal Macrophages in Age-related Susceptibility to JEV in Mice

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Abstract Attempts were made to elucidate the role of resident peritoneal macrophages in age-dependent response of mice to intraperitoneal inoculation of Japanese encephalitis virus (JEV) by treating them with anti-macrophage serum and silica prior to virus challenge. Higher mortality, and lower average survival time, were observed in anti-macrophage serum and silica treated mice over the controls. Further, increased viremia and higher virus content in the brain were observed in anti-macrophage serum treated mice of 14 and 21 days. In vitro studies with cultured macrophages from different age groups showed that anti-macrophage serum impairs the physiological functioning of the macrophage population.

The age-specific response of mice to Japanese encephalitis virus (JEV) inoculation by peripheral route is well-known. Kulkarni and Goverdhan reported that the development of resistance after 14 days of life in Swiss mice (NIV strain) to JEV challenge by intraperitoneal (ip) route is due to maturation of immunoresponsiveness. In the light of the observations with regard to virus-macrophage interaction in other virus systems such as herpes simplex, rabies etc., attempts were made to understand the macrophage involvement in age-related susceptibility of mice to JEV.

Anti-macrophage serum (AMS) raised in rabbits was used for a selective blockade of macrophage functions prior to challenge with JEV by ip route. The activity of AMS was compared with silica-treated mice since silica is known to be cytotoxic for macrophages. The schedule followed for AMS, silica and virus challenge in different age groups is given below:

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Age of mice (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>(1) Anti-macrophage serum</td>
<td>−</td>
</tr>
<tr>
<td>(ip route)</td>
<td></td>
</tr>
<tr>
<td>(2) Silica (ip) mg</td>
<td>10</td>
</tr>
<tr>
<td>(3) Virus LD&lt;sub&gt;50&lt;/sub&gt;/0.03 ml</td>
<td>10⁴</td>
</tr>
</tbody>
</table>

AMS and silica injected 2 hours before virus challenge
AMS and silica treatment significantly increases the mortality and reduces the size average survival time in relation to age (Table 1).

Table 1. Effect of AMS and silica in different age groups

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>AMS treated</th>
<th>Control</th>
<th>Silica treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mty% AST*</td>
<td>Mty% AST*</td>
<td>Mty% AST*</td>
<td>Mty% AST*</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>100</td>
<td>4.5</td>
<td>94</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>5.0</td>
<td>100</td>
<td>6.0</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>5.0</td>
<td>27.3</td>
<td>7.5</td>
</tr>
<tr>
<td>21</td>
<td>66</td>
<td>8.0</td>
<td>25</td>
<td>11.0</td>
</tr>
</tbody>
</table>

* = average survival time

The survival time observed in AMS treated and control mice of 12 and 21 days was analysed by factorial completely randomised design to know whether age and AMS act independently or synergistically. AMS treatment significantly affects age in relation to its resistance to JEV, while age, in turn, responds to AMS action too. However, there is no interaction between age and AMS activity (P < 0.01). AMS was further used to study the virus clearance from blood and virus titres in the brain. AMS treated mice of 12 and 21 days showed enhanced viremia (Table 2) as compared to the controls.

Table 2. Viremia in AMS treated mice

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>12 PID</th>
<th>Control</th>
<th>AMS* 1 dose</th>
<th>AMS* 2 doses</th>
<th>AMS* 3 doses</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.6**</td>
<td>1.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>1.8</td>
<td>0.3</td>
<td>2.6</td>
<td>2.6</td>
<td>nil</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>traces</td>
<td>nil</td>
<td>nil</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = 1st dose of AMS (0.3 ml) given 2 hours before virus challenge. Subsequent doses at 24-hour intervals. Heparinized blood was titrated intracerebrally in the infant mice of 2-3 days.

** = LD<sub>50</sub>/0.02 ml

Virus titres at the peak of sickness (moribund stage) in 12-day old mice were 10<sup>8</sup> and 10<sup>3</sup> LD<sub>50</sub>/0.03 ml in AMS treated and control mice, respectively. AMS treatment showed a dose-dependent potentiation of viremia
Peritoneal macrophages in age-related susceptibility to JEV in adults (Table 2). However, the virus titres in the adult (21 days) brain ranged from $10^5$ to $10^6 \text{LD}_{50}/0.03 \text{ml}$ (control $10^{1.5} \text{LD}_{50}$) and no exaltation of the virus was noticed despite multiple doses of AMS.

The increased susceptibility of mice to JEV challenge subsequent to AMS treatment observed in the present study suggests that the macrophages play an important role in the age-dependent resistance. This may be partly due to the extrinsic activity of the macrophages which helps in the clearance of virus from the host system and thereby allowing the central nervous system to remain intact. Similar results have been reported with herpes simplex and rabies virus in mice. However, it was observed that resident peritoneal macrophages of different age groups of mice did not support the replication of JEV (unpublished). Similar observations with several other members of alpha viruses and flaviviruses, including JEV were reported which suggests that the intrinsic interaction with virus may be lacking under cultured conditions.

The effect of AMS on the cultured macrophages derived from different age groups was studied to understand its action on the physiological properties such as attachment, phagocytosis, etc. In addition to the loss of attachment and spreading, the macrophages of treated animals exhibited lack of phagocytosis of *Staphylococcus albus*, as opposed to controls which were treated with normal rabbit serum. The incorporation of AMS in the medium (Eagle's minimum Essential Medium with 5% goat serum) used for culturing macrophages from 12-day and 21-day old mice also inhibited the attachment and phagocytosis. It was observed that AMS treatment even for 30 min. affects the phagocytosis and no bacteria could be seen in the cytoplasm.

Thus it is apparent from the above observations that macrophages constitute a barrier to ip inoculation after 14-16 days of life. Christopher *et al.*, reported the defect in antigen presentation of neonatal mice, although the distribution of Fc and C3 receptors was similar to adult. Whether such type of mechanism is involved in JEV-macrophage interaction needs to be investigated.

The authors are thankful to Dr. S. N. Ghosh for his valuable guidance and to Dr. R.P. Dolankar for the statistical analysis.

REFERENCES


5 Zisman, B. 1970; Selective effect of anti-macrophage serum, silica and ALS on pathogenesis of herpes simplex virus infection of young adult mice. *Journal of Immunology*, 104: 1155-1159.


Persistence of Japanese Encephalitis Virus in Tissue Culture

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Abstract Porcine kidney (PS) cells persistently infected with Japanese encephalitis (JE) virus have been maintained for the past 20 months. The cells have undergone 70 passages and are totally refractory to superinfection with the parental virus. Over 60% of the cells are positive for antigen by fluorescent antibody (FA) test. High HA/pfu ratio in culture supernatant suggests presence of defective interfering particles. Eight temperature sensitive mutants (ts) at a frequency of 12% have been isolated. Interferon does not appear to play any role in the maintenance of persistence.

PS cells were infected with a plaque purified P20778 strain (a human isolate) of JE virus at a multiplicity of ten. A few cells which survived the infection produced colonies after incubation at 37°C. A continuous cell line was obtained from the surviving cells. After establishment of the culture, the cells were passaged at a weekly interval and underwent more than 70 passages. The cells were cryopreserved after every 10 passages.

Persistently infected (PI) cells at low passage (P-12) were shown to be 100% positive for JE antigen by direct FA test and about 60% positivity at high passage level (P-65). Infectious centre assay, however, revealed that almost 100% of the cells harboured infectious virus at both high and low passage levels.

Superinfection studies with five strains of JE virus, five strains of West Nile virus and Dengue 1, 2, 3 viruses showed total inhibition of plaque formation on PI monolayers at P-12, P-35, P-50 and P-65. No such restriction was observed with completely unrelated viruses, viz. Sindbis, Semliki forest, Chikungunya, Polio-1, Herpes simplex type-1, Vaccinia and Chandipura (Rhabdo).

Culture fluid from PI culture was centrifuged at 100 000 x g and the pelleted virus was used to reinitiate the persistence in fresh PS and Vero cells. Persistence was immediately obtained in both cell types. Surviving Vero cells (nonproducers of interferon) were passaged two times at a weekly interval, at which time 100% cells showed JE antigen by direct FA test and the cells were also refractory to superinfection with the parental virus P20778.

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Culture fluids from high and low passage PI cells and from JE virus infected PS cells were treated with protamine sulfate and assayed simultaneously for haemagglutination (HA) and plaque formation on normal PS cultures. High HA/pfu ratio obtained from PI culture fluids (100-fold more than JE virus-infected PS culture fluid) suggested the presence of defective interfering particles.

Culture fluids from PI cultures between P-33 and P-35 were used to infect fresh PS monolayers after appropriate dilutions so as to get single plaque/0.2 ml. Sixty-five single, well-isolated clones were collected and tested for their ability to plaque at 37°C and 39.5°C. Eight tentative ts mutants scored from this initial screening procedure were grown in PS cultures at 31°C. The stocks were assayed by titration in PS cells at 31°C, by 37°C and 39.5°C. Similar titres were obtained with cultures incubated at 31°C and 37°C; but 39.5°C/31°C ratios for ts mutants ranged between $10^{-2}$ and $10^{-6}$ confirming their ts phenotype.

From the present studies it appears that the persistence has been maintained by DI particles and ts mutants.

In vivo studies, polypeptide analysis of PI cultures and characterization of ts mutants are in progress.
IgM Antibody Capture ELISA for Rapid Diagnosis of Japanese Encephalitis

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Abstract Sera from Japanese encephalitis (JE) patients were tested with an IgM antibody capture ELISA. The assay used five reagents: antihuman IgM, test serum, HKC JE virus antigen, JE antibody, enzyme conjugate and substrate. Positive JE IgM were detected in 53.7% of the patient sera collected at the time of hospital admission. The results of MAC ELISA were significantly correlated with that of CF or HI test. The absorbance value of a single dilution of test serum was closely correlated with the endpoint titre of serial dilution. MAC ELISA generally showed a high degree of specificity and is useful for the rapid diagnosis of JE.

JE is a public health problem of increasing concern to countries of South-East Asia and the Western Pacific. Essentials to the institution of an accurate and timely surveillance is the ability to diagnose JE and to recognize cases early. The conventional serological techniques of haemagglutination inhibition (HI), complement fixation (CF) and neutralization (N) are useful, but demonstration of HI or N antibody needs paired serum causing delays in diagnosis. The presence of CF antibodies is an indication of recent infection but it appears rather late and some patients never develop detectable CF antibodies. In recent years, IgM antibody capture ELISA (MAC ELISA) has been proven especially useful for rapid diagnosis of viral diseases. In this paper, we report the application of this technique to the rapid diagnosis of JE in Shanghai in 1984.

MAC ELISA TEST PROCEDURES

(1) Polystyrene plates were coated with antihuman IgM diluted 1:1000.

(2) Serial two-fold dilutions of test patient sera (from 1:100 to 1:25600) were added. A single dilution of test serum (1:800) was added in some tests. Controls in each test included known positive and negative human sera and normal saline.
(3) JE virus antigen (concentrated vaccine, P3 strain) was added. Heterologous flavivirus antigens (dengue 1-4) were used in some tests.

(4) Anti-JE virus antigen conjugated with HRP was added.

(5) Substrate (p-nitrophenyl phosphate) was added.

The absorbance values at 492 nm were determined with a Titre Tek Multikan apparatus, the mean value of duplicate wells of each dilution of test serum (p) was divided by the value of the corresponding dilution of a negative control serum (N). We postulated that the positive criteria for serodiagnosis on JE by serial dilution endpoint test would be 1:800, and a P/N ratio of 2.0 was considered positive in single dilution test.

RESULT

(1) The total positive rate of JE IgM antibodies of 54 patients was 53.7% (29 patients) using the first serum specimen collecting at the time of hospital admission. All the results had been confirmed by CF or HI test.

(2) Comparison of the results of endpoint of serial dilutions with the single dilution of patient sera indicated they were closely related, so the JE IgM antibody titre can be estimated by a single dilution serum.

(3) A total of 163 sera specimen collected from healthy blood donors, or non-infectious disease patients in the same hospital, were all JE negative (titre 1:20). Some positive sera had been tested simultaneously against JE negative virus antigen and heterologous flavivirus of the same subgroup, i.e. dengue 1-4. Little overlap existed, but it could apparently be distinguished.

Our preliminary results indicated that the MAC ELISA is sensitive, accurate and most specific. As it avoids competition between IgG and IgM and the problem of the presence of rheumatoid factor, it offers various advantages to provide a practical basis for the rapid diagnosis of JE. However, a further standardization of all reagents, test procedures and positive criteria is necessary.
Haemorrhagic Fever with Renal Syndrome: A Singapore Case

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INTRODUCTION

Haemorrhagic fever with renal syndrome (HFRS) came to the attention of western medicine during the Korean War when it was a major source of morbidity and mortality among the United Nations troops in Korea. The disease, hitherto unreported in Western medical literature, was characterized by fever, headache, back pain, abdominal pain, a flushed face, petechial haemorrhagic manifestations, shock and acute renal failure in its classical form, with a mortality of 10 to 15%. The less severe form of the disease presents as a non-haemorrhagic flu-like illness with proteinuria and azotemia. The disease is now known as haemorrhagic fever with renal syndrome (HFRS), a term recommended by a WHO working group on HFRS.

The causative agent, known as the Hantavirus, was first isolated from the lung tissue of the field mouse, Apodemus agrarius, which is the principal reservoir for HFRS in Korea and rural China. Urban rats and laboratory rats have also been shown to transmit the disease. Infection by the respiratory route from aerosols is believed to be the major route of transmission of the disease. No secondary cases or evidence of person to person transmission has been observed.

Geographically, HFRS is endemic across much of the Eurasian land mass (Russian Europe, Soviet Far East, Korea, Japan, China, Scandinavia and Eastern European countries). Immunofluorescent antibodies against the Hantavirus have been demonstrated in urban rats in areas where the human disease has not been reported, such as port areas in the United States (New Orleans, Philadelphia and Houston) and in Japan (Kobe and Yokohama). Focal clustering of seropositive rats was observed in these studies. Evidence of rodent infection was also found in Thailand, Malaysia, Philippines and Taiwan. The case described below is the first reported in Singapore.
CLINICAL HISTORY

The patient is a 36-year old Chinese female who complained of fever, vomiting and lethargy in early October 1984 and was referred by her family physician to a district hospital in Singapore because of haematuria. Physical examination showed pallor, minimal ankle oedema but no skin rash or petechiae. She was then afebrile but her blood pressure was 150/95 mm Hg. Urine microscopy showed 6 to 8 RBC, 4 to 5 WBC and 4 to 5 epithelial cells per high power field. There was mild albuminuria. Her haemoglobin was 9.7 g %. Her total white cell count and differential count were within normal limits. Serum urea on admission was 146 mg/dl, falling to 138 mg/dl 3 days later (normal range 10-20 mg/dl). The serum creatinine was 9.7 mg/dl (normal range 0.5-1.6 mg/dl) while creatinine clearance was 4 ml/min (normal range 70-150 ml/min). The serum electrolytes were within normal limits. Her hypertension was controlled by a methyldopa 125 mg twice daily and she was given resonium. A 15 mg three times daily since admission for 4 days, after which she was discharged. She was an asymptomatic since then. Diagnosed as "chronic renal failure", she was referred to the specialist clinic in Singapore General Hospital for further management in December 1984. Her serum creatinine dropped to 5.6 mg/dl. An intravenous programme showed a small smooth kidney with good excretory function. Renal biopsy was not done.

During her acute illness in October, she was included in a sero-survey for immunofluorescent antibody against Hantaan virus and Puumala virus (Nephropathia epidemica agent), using the 76/118 strain in A546 cell culture and Sotkamo strain in Vero-E6 cell culture. Her IF antibody titre was 1 to 640 (acute phase) against Hantaan virus, but less than 16 against Puumala virus, strongly suggestive of Hantaan virus infection.

This patient worked as a quality controller in an electronic factory. She lived in a two-room flat on the ground floor of a public housing estate in Toa Payon, a new town. She reported that rat infestation had been a problem in her neighbourhood. From 19 to 26 June 1984 she visited her sister's family in a rural village in Perak, West Malaysia with rubber plantations and a considerable rat population in the vicinity.

DISCUSSION

HFRS is endemic in most parts of Eurasia. Recent serological studies have shown evidence of human infection in North America as well. However, human cases have not been reported in South-East Asia, despite serological evidence of rodent infection. Since domestic rats and field rodents, the principal reservoir for the disease, are widely distributed in South-East Asia, HFRS is unlikely to be non-existent here. Its apparent absence probably reflects unawareness by clinicians of this "new disease", which may
have been labelled as "acute nephritis" or "chronic renal failure" (as in this patient), terms which shed no light on the etiology of the patients' illness. The clinical manifestation of HFRS in South-East Asia may well be different from that of the classical form with overt hemorrhagic manifestation, severe shock and high mortality. This case resembles the urban form found in cities of Japan and Korea and the provinces of Henan and Shanxi in Northern China, which runs a milder course resembling influenza, often without hemorrhagic phenomena but with evidence of renal dysfunction. The benign course of this patient, with gradually improving renal function, agrees with this clinical entity. It is not known whether her infection is acquired in her home environment in Singapore or during her visit to Malaysia, since rat infestations were reported in both places. The incubation period for HFRS is 1 to 8 weeks, making it difficult to determine the time of exposure to the infectious agent.

Rodents have been a perpetual enemy of mankind, spoiling our food and afflicting human with numerous diseases directly or indirectly. HFRS is a new but important addition to the list. Since man-to-man transmission has not been reported, effective control of the rodent reservoir in rural and urban settings, should reduce its incidence in endemic areas and lower the risk of its introduction in non-endemic areas. The isolation of Hantaan virus by one of us represents an important milestone in HFRS research. Since then there has been much advance in the knowledge of the disease. However, many questions remain unanswered in respect of antigenic variations of Hantaan and its related viruses, and of the geographical distribution and clinical patterns of HFRS. Hantavirus is a new genus name for Hantaan and related viruses and Seoul virus, which was isolated from urban rats in 1980, was registered in the arbovirus catalogue in 1985. It can be assumed that a Seoul-virus-like virus in Singapore could be the causative agent of the HFRS case in Singapore and it remains to be studied. Further research in these areas and in the bionomics of the rodent reservoir should be useful in the successful control of the disease.

REFERENCES


Isolation of Seoul-like Viruses from Lungs of *Rattus* Captured in Hong Kong

Ho Wang Lee*, Pyung-Woo Lee* and W. K. Chang**

*The Institute for Viral Diseases, Korea University, National College, Seoul, Korea
**Queen Mary Hospital, Hong Kong

Six strains of Seoul-like viruses were isolated in Vero E6-cell cultures inoculated with 10% lung suspensions of *Rattus* captured in Hong Kong metropolitan areas.

The recent isolations of Hantaan related viruses from wild urban rats trapped in Korea¹, Japan², the People's Republic of China³,⁴ the United States⁵,⁶, Egypt⁷ and Brazil⁸ indicate the rats may also serve as the primary reservoirs of Hantavirus in urban centers.

Recently, these (*Rattus*-borne) viruses were named as Seoul virus⁹ and suggested to be classified as a different serotype of Genus Hantavirus, including Hantaan, Puumala and Prospect Hill virus.¹⁰

Clinically inapparent infections characterized by transient viremia, virus persistence in tissues and high levels of fluorescent and neutralizing antibodies were developed in experimental rats inoculated with Seoul virus.¹¹

This is the first isolation of *Rattus*-borne virus (Seoul virus) from urban rats caught in an island in the Western Pacific Region.

The studies on relation with other Hantaviruses, especially Seoul virus and the biological characterization of these isolates are in progress.

REFERENCES


<table>
<thead>
<tr>
<th>Virus Name</th>
<th>Abbreviation</th>
<th>Antigenic group</th>
<th>SALS</th>
<th>Taxonomic status</th>
<th>Hantavirus proposed</th>
<th>Rating</th>
<th>Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seoul Virus</td>
<td>SELV</td>
<td>Bunyaviridae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**I. FULL VIRUS NAME AND PROTOTYPE NUMBER**
Seoul Virus — Strain 80-39

**II. ORIGINAL SOURCE**
Isolated by Ho Wang Lee et al (1) at Seoul, Korea

**III. METHOD OF ISOLATION AND VALIDITY**
Inoculation: Day 23 Month April Year 1980

**IV. Animal**
Embryonated egg

**V. Route**
Intramuscular

**VI. Resolation**
Yes x No Not tried Other reasons

**VII. Homologous antibody formation**
Source animal (See II(2)): Yes x No Not Tested

**VIII. Test used**
HI CF NT Other Indirect FAT
IV. (1) VIRUS PROPERTIES: Physicochemical: RNA, DNA, Single strand, or Double

(2) Pieces 3 (22) Infectivity, Sedimentation coefficient(s) S.

(3) Percentage wt. of virion protein, lipid, carbohydrate

(4) Virion polypeptides: Number 4 Details Nucleocapsid protein

(5) 50,000 MW; glycoproteins (G1) 76,000 MW (G2) 54,000 MW

(6) (L) approx. 200,000 MW

(7)

(8) Non-virion polypeptides: Number Details

(9) 

(10) Virion density: 1.18-1.20 in sucrose Sedimentation coefficient (S)

(11) Nucleocapsid density in Sedimentation coefficient (S)

(12) Stability of infectivity (effects): pH Maximum stability at pH 7.6, inactivated completely at pH 4, 0

(13) Lipid solvent: (ether) 20% sensitive After treatment titer 101.0/0.5 ml Control titer 105.8/0.5 ml

(14) (chloroform) 5% sensitive After treatment titer 101.0/0.5 ml Control titer 105.8/0.5 ml

(15) Detergent: (deoxycholate) 0, 1% sensitive After treatment titer 101.0/0.5 ml Control titer 105.8/0.5 ml

(16) Other (formalin, radiation) Inactivated with 0.4% formalin, 70% ethanol, 0.5% iodine & UV radiation

(17) Virion morphology: Shape Round Dimensions 90-140 nm

(18) Mean 100 nm; range 90-140 nm; how measured E.M. (4-5)

(19) Surface projections, envelope Surface projections and enveloped

(20) Nucleocapsid dimensions, symmetry

(21) Morphogenesis: site of constituent formation in cell Cytoplasm

(22) Site of virion assembly

(23) Site of virion accumulation

(24) Inclusion bodies Other

(25) Hemagglutination: Yes No Not tried Antigen source nb mice brain (6) & Vero E6

(26) Erythrocytes Goose; pH range 5.8-6.4; pH optimum 6.0-6.2 cell cultures

(27) Temperature optimum 37°C range 20-37°C remarks

(28)

(29) Serologic methods recommended Neutralization test, plaque reduction test, IFAT and Elisa Test
V. ANTIGENIC RELATIONSHIP AND LACK OF RELATIONSHIP TO OTHER VIRUSES:

Seoul virus infected tissue culture cells and sections of rat lungs react extensively by IFAT with convalescent sera from HFRS patients in Korea, Japan, China, USSR and nephropathia epidemica in Scandinavia. Seoul virus cross-react with Hantaan virus and Prospect Hill virus by IFAT but differentiable by plaque reduction test, Elisa test and passive haemagglutination inhibition test. Seoul and Hantaan viruses are different serologically from monoclonal antibodies made with Hantaan virus antigen (BDOI-BBO8) by IFAT.

Antigenic relations of HFRS-related viruses: 80 percent PRNT titres, PRNT’s were performed with rat antisa (as described in the legend to Table 2) and cell culture-adapted viruses. Approximately 100 PFU of virus were incubated with two-fold dilutions of sera for 1 hour at room temperature before inoculation of 25-cm² flasks. Inoculated cells were further incubated for 1 hour at 37°C before addition of an overlay of EMEM containing 0.6 percent agarose (Seakem ME), 10 percent heated fetal bovine serum (FBS), 2mM L-glutamine (Gibco), penicillin (100 U/ml), streptomycin (100 Ug/ml), and Fungizone (0.5 Ug/ml: Gibco). Following incubation for 7 to 14 days after infection a second overlay identical to the first except for a reduced FBS concentration: 0.167 mg/ml was applied. Plaques were counted as they became visible 1 to 6 days after addition of the second overlay. Titres were expressed as the reciprocal of the highest dilution of antibody resulting in greater than 80 percent reduction of approximately 100 plaques. Asterisks indicate titres.

<table>
<thead>
<tr>
<th>Serum pools</th>
<th>HTN</th>
<th>LEE</th>
<th>UR</th>
<th>TCH</th>
<th>GP</th>
<th>SR-11</th>
<th>PH</th>
<th>PA</th>
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<tbody>
<tr>
<td>HTN</td>
<td>4000</td>
<td>4000</td>
<td>0</td>
<td>80</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>LEE</td>
<td>4000</td>
<td>2000*</td>
<td>20</td>
<td>160</td>
<td>160</td>
<td>200</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UR</td>
<td>2000</td>
<td>80</td>
<td>4000</td>
<td>16000</td>
<td>16000</td>
<td>8000</td>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td>TCH</td>
<td>2000</td>
<td>200</td>
<td>4000</td>
<td>32000</td>
<td>8000</td>
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<td>200</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1280</td>
</tr>
</tbody>
</table>

Table: Isolation of Seoul-like viruses from Rattus

HTN = Hantaan Virus 76-118
LEE = Human isolate from Korean
UR = Seoul Virus
TCH = Tchoupitoulas
PG = Girard Point
SR-11 = Sapporo Rat
PH = Prospect Hill
PA = Puumala
VI (1) BIOLOGICAL CHARACTERISTICS: Virus source (all VERTEBRATE isolates):
(2) Blood, lungs, kidneys, parotid glands, saliva, urine and feces of infected urban rats (1)
(3) __________________________________________________________
(4) Lab Methods of Virus Recovery (ALL ISOLATIONS) 1) Vero E6 cells, 2) Laboratory rats and
(5) __________________________________________________________
(6) Susceptibility of Cell Culture Systems:

<table>
<thead>
<tr>
<th>Cell system</th>
<th>Virus passage history</th>
<th>CPE</th>
<th>PLAQUES</th>
<th>Evidence of Infection</th>
<th>Growth Without CPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day (c)</td>
<td>Exent (d)</td>
<td>Titer TCD50/ml (e)</td>
<td>Day (c)</td>
</tr>
<tr>
<td>Vero E6 cells (5)</td>
<td>Vero-P2</td>
<td>7-10</td>
<td>0</td>
<td>$10^{6.3}$ IFID*</td>
<td>7</td>
</tr>
<tr>
<td>A549 cells (11)</td>
<td>Vero-P2</td>
<td>7-10</td>
<td>0</td>
<td>$10^{8.3}$ IFID*</td>
<td>9</td>
</tr>
</tbody>
</table>

*Fluorescent foci forming units/ml.
IX. (1) EXPERIMENTAL ARTHROPOD INFECTION AND TRANSMISSION

<table>
<thead>
<tr>
<th>Arthropod species &amp; virus source (a)</th>
<th>Method of Infection</th>
<th>Incubation period (c)</th>
<th>Transmission</th>
<th>Assay of arthropod log 10/ml (e)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Feeding</td>
<td>Injected</td>
<td>Days °C</td>
<td>Host</td>
</tr>
<tr>
<td></td>
<td>log 1/ml (b)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X. (1) HISTOPATHOLOGY: Character of lesions *Haemorrhagic lesions are found in the pituitary, the right tricuspid valve, and the kidney.*

(2) Intense capillary congestion and small areas of necrosis in the viscera (M). Hemorrhagic lesions in the viscera and encephalitis in the nb mice and the nb rats by inoculation of the virus (LV).

(3) Inclusion bodies: Cytoplasmic: (M) None (LV) None Intranuclear: (M) None (LV) None

(4) Organs-tissues affected Heart: haemorrhagic in the right atrium, kidney: severe congestion of medulla

(5) Pituitary gland congestion and necrosis in the anterior lobe. Lung: oedema (M)

(6) Brain: encephalitis. Viscera: haemorrhagic lesions in nb, mice and rats (LV)

(7) Category of tropism: Kidney & parotid glands (M), lung, kidney and parotid glands (LV)

XL. (1) HUMAN DISEASE: In nature (S) x (R) x _ Death: (S) x (R) x _ Residua: (S) x (R)

(2) Laboratory infection: Subclinical: (S) x (R) x _ Overt Disease: (S) x (R) x _

(3) Clinical manifestations: Fever, headache, vertigo, pharyngeal injection, nausea, rash, backache

(4) Proteinuria, hypostenuria, polyuria, hematuria, increased E.S.R., a typical lymphocytes, oliguria

(5) Azotemia, constipation, hypotension, leukocytosis (10,000/mm³), thrombocytopenia (100,000/mm³)

(6) Category Haemorrhagic fever with renal syndrome No. of cases 70-100 cases/year in Seoul, 126 cases in Osaka

XII. (1) GEOGRAPHIC DISTRIBUTION:

(a) Known (virus) Korea and Japan. Similar illness exist in China, USSR, Eastern and Northern Europe (18-21).

(b) Suspected (antibody) Hong Kong, Philippines, Malaysia, Singapore, Thailand, Australia, Fiji, Hawaii, U.S.A., Brazil, Belgium, Greece, Egypt, Sudan and Uganda (some data are not published).
XIII.(1) REFERENCES:

XIV.(1) Remarks:

Synonyms of human disease: Korean Haemorrhagic Fever, Epidemic Haemorrhagic Fever, Songa Fever, Haemorrhagic Nephroso-Nephritis, and Nephropathia Epidemica serologically related and clinically similar diseases are referred to as Haemorrhagic Fever with Renal Syndrome.
The Prevalence of HFRS Antibody in the renal/hepatic Dysfunction Group in Japan

Takashi KITAMURA, Toshihiko KOMATSU, Sadashi SHIGA, Shigeru MORIKAWA, Teruko KOHARA, Momoko OGATA, Yoriyuki AKAO

Division of Poxviruses and Special Pathogens, National Institute of Health, Musashimurayama, Tokyo 190-12, Japan.

For the purpose of assessing the prevalence of antibody against HFRS or related viruses among Japanese populations, we have assayed the indirect fluorescent antibody (IFA) positive ratios of serum collected from various population groups. Healthy populations are those collected by the WHO Serum Reference Bank in Tokyo in 1982 from 13 prefectures randomly distributed between Hokkaido, the northernmost part of Japan, to Kyushu Island in the south. The renal/hepatic dysfunction group is composed of those which showed pathological value in any of GOP, GPT, LDH or CPK test at the clinical laboratories, irrespective of their clinical manifestations. The renal dialysis group is composed of individuals subjected to the dialysis therapy at least once a week. Both groups were composed of approximately equal numbers of sera collected in the Tokyo and Osaka areas. The suspected leptospirosis group is composed of those suspected of clinical leptospirosis but showing negative results of leptospira antibody tests. IFA titre was assayed by the established procedure against the antigen slides of VERO-E6 cells infected with SR-11 strain of HFRS virus; a serum showing an antibody titre not lower than 1:16 was taken as positive.

From a total of 5073 sera of healthy population, 48 were found positive (0.95%); no geographical accumulation of the positive cases was observed. Antibody titres ranged between 1:16 and 1:128. The antibody prevalence of 0.95% may be regarded as the background rate among Japanese healthy population.

Antibody prevalence in the renal/hepatic dysfunction group was 25/1004 (2.49%) in Tokyo and Osaka areas. The percentages were 1.75% and 3.50%, respectively, suggesting that HFRS or related virus might have caused a typical symptom with renal or hepatic involvement and its incidence may be higher in the Osaka than in the Tokyo area. In the renal dialysis group, antibody prevalence was 5/283 (1.77%); that was significantly higher than the background level, suggesting that a small number of persistent renal dysfunction calling for dialysis may be related to the HFRS or
related virus infection. The suspected leptospirosis group was not sufficiently big in number but showed a significantly high prevalence of 1/30 (3.33%). Antibody prevalence in the suspected leptospirosis group is under continuous surveillance.

Those results strongly suggest that HFRS-related viruses prevalent in Japan may be causing atypical symptoms with, most probably, renal or hepatic involvements.
Epidemiological Studies of Haemorrhagic Fever with Renal Syndrome (HFRS) Virus Infection among Urban Rats in Hokkaido, Japan

Jiro ARIKAWA, Ikuo TAKASHIMA and Nobuo HASHIMOTO

Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan.

Seroepidemiological studies of haemorrhagic fever with renal syndrome (HFRS) virus infection among urban rats in Hokkaido, Japan were carried out by the indirect immunofluorescent antibody test (IFAT). An urban rats colony that was seropositive to SR-11 strain of laboratory rat origin HFRS virus was demonstrated in February 1983 at a dumping ground area of Kami-iso Town, which is located 10 km west of Hakodate port (Table 1). The causative virus, named Kami-iso 262 (KI-262) strain, was isolated from a sero-positive rat's lung tissue using Vero-E6 cell culture.

Table 1. Survey at Kami-iso Town

<table>
<thead>
<tr>
<th>Date of Survey</th>
<th>Younger than a month*</th>
<th>6 months or older*</th>
<th>Total serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. serum</td>
<td>No. pos. (%)</td>
<td>No. serum</td>
</tr>
<tr>
<td>Feb. 1983</td>
<td>0</td>
<td>0 (0.0)</td>
<td>6</td>
</tr>
<tr>
<td>Aug. 1983</td>
<td>20</td>
<td>0 (0.0)</td>
<td>17</td>
</tr>
<tr>
<td>Oct. 1983</td>
<td>18</td>
<td>0 (0.0)</td>
<td>9</td>
</tr>
<tr>
<td>May 1984</td>
<td>2</td>
<td>1 (50.0)</td>
<td>1</td>
</tr>
<tr>
<td>July 1984</td>
<td>26</td>
<td>4 (15.4)</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>66</td>
<td>5 (7.6)</td>
<td>42</td>
</tr>
</tbody>
</table>

*Age of the rats were estimated by eye lens weight method.
Although the KI-262 strain was shown to belong to rat origin virus group by the cross neutralization test (Table 2), slightly different antigenicity was observed among them.

Table 2. Antigenicity Variations

<table>
<thead>
<tr>
<th>Immune Serum</th>
<th>SR-11</th>
<th>TB-314</th>
<th>KI-262</th>
<th>HTNV</th>
<th>PHV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR-11</td>
<td>1,280</td>
<td>1,280</td>
<td>320</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>TB-314</td>
<td>1,280</td>
<td>1,280</td>
<td>320</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>KI-262</td>
<td>640</td>
<td>640</td>
<td>1,280</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HTNV</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>160</td>
<td>10</td>
</tr>
<tr>
<td>PHV</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>640</td>
</tr>
</tbody>
</table>

Titres were expressed as the reciprocal of the highest dilution of immune serum, resulting in greater than 50% reduction in the number of infected cell foci in the control wells.


Four additional follow-up studies were conducted at the dumping ground area until July 1984 (Table 1). Higher positive ratios were observed in the spring and winter seasons than in the summer and fall ones. In addition, a significantly high proportion of positive cases was observed among adult rats (six months or older) than among younger ones. The results suggested that the causative virus was not readily transmitted from one infected rat to another. Close contact of rats with an infected animal in a contaminated nest during the cold season appears to be necessary for the transmission of the virus.

REFERENCE

Direct Detection of Hantavirus Antigen

CHEN Boqian*, ZHOU Guofang*, LIU Qingzhi*, MENG Qingrong*,
WU Maiying*, WANG Shiming**, XU Mengsu**, ZHAO Jing**,
JIANG Yang**, ZHAO Chunyuan**, WANG Yingchun**,
ZHAO Ruqin**, LU Zaiying**, LI Zhongxun**

*China National Centre for Preventive Medicine, Institute of Virology
**Shenyang Infectious Disease Hospital, Department of Virology

Direct detection of the antigen of Hantavirus in the cells of urine and white blood cells obtained from 138 HFRS patients using HFRS Mc Ab 25-1 FITC by direct IF test was carried out.

The positive rate of the antigen in white blood cells and cells of urine were 82.6% (114/138) and 71% (98/138) respectively. In the same patients the positive rate of IF antibody to Hantaan virus was 81.2% (112/138). However, detection of the antigen from 48 healthy persons and other kinds of disease were negative.

The positive antigen was found mainly within 10 days, during fever and oliguric phases of the illness, but the high positive rate of IF antibody was found mainly after 10 days. It also found that adding the results of detection of antigens and antibodies, the diagnosis rate can raise up to 98.5%.

Antigen detection from white blood cells and urine was easier to carry out than the antibody detection as the antigen appears earlier than the antibody. So detection of antigen can probably be used for early diagnosis of HFRS.

REFERENCE

Protective Role of Passive Administration of Antibody against HFRS

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Rats infected with HFRS virus show high prevalence of viral antigen in lung tissue despite high titres of IFA antibody in their blood, puzzling us with the protective role of circulating antibodies. As has been reported earlier (Arch. Virol, in press), sensitivity of rats to HFRS virus infection is age-dependent, i.e. rats inoculated within three weeks of age can grow to be a persistent virus shedder (sensitive age), while those inoculated after three weeks of age can develop antibody response but not have the transmissible virus in tissues (semi-sensitive age).

Three week old rats were administered intraperitoneally (ip) with 0.5 ml each of rat immune serum with an IFA antibody titre of 1:8000, to be followed by subcutaneous inoculation (sc) of $10^4$ TCID$_{50}$ of SR-11 strain of HFRS virus 24 hours later. The control group of rats of the identical age without immune serum administration had IFA antibody titres 1:256-512 and 1:4000 after 10 and 21 days following inoculation, respectively, whereas the passively immunized group had only declining antibody titres of 1:128, 1.64-128 and 1:32-64 after 0, 10 and 21 days respectively, suggesting that the latter group could not develop immune response to newly inoculated virus, i.e. the failure of infection.

Newborn rats, 24 hours after birth, were administered ip with 0.2 ml each of the same immune serum, to be followed by inoculation of $10^4$ TCID$_{50}$ of SR-11 virus. IFA antibody level six weeks after infection were 1:8000-16000 in the control group of the same age in contrast with those 1:16-32 in the passively immunized group, suggesting that virus infection was blocked in the latter group. Average body weight of the control group was nearly 50% of that of passively immunized group, which was comparable with the normal animals of the same age, serving as another evidence of the fact that the passively immunized animals were completely protected from the virus infection. The transmission of the virus to the cage-mates of the same age (virus shedding) was also negative in the passively immunized group. These findings are important in that they have demonstrated that passive administration of antibody could protect experimental rats even at the sensitive age.