EPIDEMIOLOGICAL STUDIES ON JAPANESE ENCEPHALITIS IN KYOTO CITY AREA, JAPAN

I. EVIDENCE FOR DECREASE OF VECTOR MOSQUITOES

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SUMMARY: Mosquito collections by using light traps have been carried out at 10 to 11 stations in Kyoto City area at intervals of about 10 days every year. Mean percent indexes (MPI), being calculated from the data of mosquito collections, were used for comparison of the annual abundance of mosquitoes. It is no doubt that Culex tritaeniorhynchus summorosus has decreased recently and this decrease is correlated with the reduction of human patients of Japanese encephalitis. Wide use of two herbicides, CNP and nitrofen, for rice plant cultivation, may probably be one of the reasons for the decrease of the mosquitoes.

INTRODUCTION

Since the Japanese encephalitis (JE) virus has been isolated mainly from Culex tritaeniorhynchus summorosus, this species of mosquitoes has been considered as the principal vector of the virus in the field. It has been also evident, as reported by Yamamoto (1971), that the epidemic scale of JE was determined by the relation between the number of mosquitoes produced in the area and the period of dissemination of JE virus in the mosquito population. When disseminations occurred coincidentally with the major peak of mosquito prevalence, a large-scale epidemic has always been experienced in that area. However, it seems probable that the marked decrease in human patients in recent years may have resulted from the reduction of the vector mosquitoes as described by Kamimura and Watanabe (1973).

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Fig. 1. Map showing the location of mosquito collection stations in the Kyoto City area. Empty and black circles represent each station in suburban and urban areas respectively.

**METHODS**

*Site of mosquito collections:* Mosquito collections started in 1965 at 10 stations in Kyoto City area. However, the stations where only small numbers of mosquitoes were caught in one year were moved to other places in the next year. Thus, 11 stations shown in Fig. 1 have been established since 1967; some stations have been moved to nearby places.

*Method of mosquito collection:* One light trap, a New Jersey type with a 60-watt electric lamp, was set at each station for collecting mosquitoes; the traps have been replaced with a Nozawa type with a 6-watt black fluorescent lamp since 1970. Whenever possible, operations were done simultaneously at all stations at interval of 10 days during the period from April to November every year. Mosquitoes collected at each station were counted by species.

**RESULTS**

*Seasonal Fluctuation of Mosquito Number*

The numbers of mosquitoes collected at each station were totalled each day. The total numbers of *C. tritaeniorhynchus* and *Anopheles sinensis* are
Fig. 2. Annual prevalence of *C. tritaeniorhynchus* (solid line) and *A. sinensis* (bloken line) as shown in the total number of the mosquitoes caught at 10 to 11 stations in Kyoto City area after 1965.
Figure 3. Annual prevalence of C. pipiens as shown in the total number of mosquitoes caught at 10 to 11 stations in the Kyoto City area after 1965.
shown in Fig. 2 and those of Culex pipiens in Fig. 3. During the initial three years from 1965 to 1967, when a large epidemic was experienced every year, C. tritaeniorhynchus was found to prevail throughout the area with a high peak in late July and a low peak between the middle of August and early September after reduction of the number of mosquitoes in early or mid August. It was found that the number of mosquitoes rapidly decreased after 1968, the number became particular low after 1970. The first peak of mosquito prevalence, which was clearly observed in late July during the initial three years, was found to be delayed in the following years, accompanied with the decrease in the number of mosquitoes.

The pattern of the seasonal prevalence of A. sinensis was similar to that of C. tritaeniorhynchus, but the reduction in number was not so marked as seen in C. tritaeniorhynchus. However, the pattern of prevalence of C. pipiens differed from those of the other two species mentioned above. This species was caught already in May as seen in Fig. 3, and prevalent with the first peak between late June and early July. Most of this species were caught at station K, where suitable environmental conditions for breeding mosquito larvae, such as stagnant drains and cess pools, were found in the neighborhood. The total numbers of mosquitoes collected at all stations may not show the abundance of this species but it is abundant at station K.

Similar situation may also influence the data of C. tritaeniorhynchus. Figure 4 shows the fluctuations of the population of this species from one station to another in 1965. Most of this species were collected at stations B, J and K in rural or suburban areas having rice fields, which were suitable for breeding larvae of this species. Thus, the total number of mosquitoes of certain species collected at all stations may not always represent the relative number of that species.

Comparison of Annual Abundance of Mosquitoes

In order to solve such a problem, mean percent index (MPI) was proposed. MPI is calculated by the following procedure. If the total number of mosquitoes caught by m-time collections at a station of i in a year of k is given by

$$X_{ik} = \sum_{j=1}^{m} x_{ijk},$$

where $x_{ijk}$ is the number of mosquitoes caught in each collection, MPI is shown in the following equation:

$$\text{MIP}_k = \frac{100}{n} \sum_{i=1}^{n} \left( \frac{X_{ik}}{X_{i0}} \right),$$

where n is the number of station and $X_{i0}$ is the total number of mosquitoes caught at each station in 1967, when the stations for the collection were being fixed.
Fig. 4. Seasonal prevalence of *C. tritaeniorhynchus* caught with light traps at each station in 1965.

**TABLE I**

*Decrease in Culex tritaeniorhynchus population in successive years after 1965 in correlation with the incidence of Japanese encephalitis*

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of patients*</th>
<th>At the 10–11 stations located in urban and suburban areas</th>
<th>At the 5 stations in only the urban area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>MPI</td>
</tr>
<tr>
<td>1965</td>
<td>44</td>
<td>9438</td>
<td>117.2</td>
</tr>
<tr>
<td>1966</td>
<td>96</td>
<td>8665</td>
<td>215.9</td>
</tr>
<tr>
<td>1967</td>
<td>41</td>
<td>11570</td>
<td>100</td>
</tr>
<tr>
<td>1968</td>
<td>10</td>
<td>2566</td>
<td>25.6</td>
</tr>
<tr>
<td>1969</td>
<td>16</td>
<td>2583</td>
<td>40.4</td>
</tr>
<tr>
<td>1970</td>
<td>0</td>
<td>544</td>
<td>9.0</td>
</tr>
<tr>
<td>1971</td>
<td>0</td>
<td>338</td>
<td>3.3</td>
</tr>
<tr>
<td>1972</td>
<td>0</td>
<td>182</td>
<td>1.5</td>
</tr>
<tr>
<td>1973</td>
<td>0</td>
<td>175</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Correlation coefficient with No. of patients: 0.808, 0.997, 0.871, 0.990, 0.980, 0.882

* Yearly numbers of the patients of clinically apparent JE were obtained from the annual municipal record of Kyoto City.
TABLE II
Relative abundance of Culex pipiens and Anopheles sinensis population in successive years after 1965

<table>
<thead>
<tr>
<th>Year</th>
<th>At the 10-11 stations located in urban and suburban area</th>
<th>At the five stations in only the urban area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culex pipiens</td>
<td>Anopheles sinensis</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>MPI</td>
</tr>
<tr>
<td>1965</td>
<td>2332</td>
<td>197.8</td>
</tr>
<tr>
<td>1966</td>
<td>7658</td>
<td>166.0</td>
</tr>
<tr>
<td>1967</td>
<td>6788</td>
<td>100</td>
</tr>
<tr>
<td>1968</td>
<td>6516</td>
<td>114.9</td>
</tr>
<tr>
<td>1969</td>
<td>3615</td>
<td>80.9</td>
</tr>
<tr>
<td>1970</td>
<td>3606</td>
<td>74.6</td>
</tr>
<tr>
<td>1971</td>
<td>3175</td>
<td>82.6</td>
</tr>
<tr>
<td>1973</td>
<td>2552</td>
<td>94.6</td>
</tr>
<tr>
<td>1973</td>
<td>1919</td>
<td>80.6</td>
</tr>
</tbody>
</table>

The result of this calculation for C. tritaeniorhynchus is shown in Table I, and that for C. pipiens and A. sinensis in Table II, including the result calculated from the data of the collections only at stations in the urban area. A marked decrease in MPI was obtained in successive years with respect to C. tritaeniorhynchus and A. sinensis; the reduction was more marked in the former rather than in the latter. However, MPI of C. pipiens showed a slight decrease for the successive years, but the decrease was seen in data from the five stations in only the urban area. C. pipiens were identified without discrimination between C. p. pallens and C. p. molestus. The former larvae breed usually in stagnant drain or cesspool in the rural or suburban area, while the latter are often found in the stagnant pool of buildings in the urban area. Therefore, these results seem to be reasonable since breeding of C. p. pallens decreased in the rural and suburban areas but that of C. p. molestus increased in the urban area.

Seasonal prevalence can be also expressed in MPI from the data of light trap collections as follows:

\[ MPI_{jk} = \frac{MPI_k}{n} \sum_{i=1}^{n} \left( \frac{X_{ijk}}{X_{ik}} \right) \]

The result of calculations from the data of C. tritaeniorhynchus is shown in Fig. 5. This expression is useful for showing annual pattern of mosquito prevalence from the data of collections at several stations.
Fig. 5. Annual prevalence of C. tritaeniorhynchus as shown in MPI calculated from the data of mosquito collections at 10 to 11 stations.

**DISCUSSION**

The seasonal prevalence of mosquitoes has been shown usually in the number of mosquitoes collected by a light trap, but the numbers were found to fluctuate day by day. Therefore, the total or mean value seems to be unfit for comparison of the annual abundance of mosquitoes. Ishii and Karoji (1975) proposed the use of collection index (total) and trap index (mean) estimated from the data obtained by light trap collections at a station, and calculated the relative error of these indices by Morisita's $I_2$ method (1959). However, it was indicated in the present study that large variations among stations in the
number of mosquitoes collected should be taken into consideration for comparison of annual abundance of mosquitoes. Besides, mosquito numbers in the period from late July to August, when the epidemic of JE would occur every year, should be emphasized in comparison of annual epidemic of JE. On the basis of this point, MPI are calculated from the totals at each station in comparison with those in the standard year. The annual number of JE patients is more closely correlated with MPI from the data at 10 to 11 stations than with the totals; the correlation coefficients are 0.997 and 0.808, respectively, as shown in Table I. High correlations of size of epidemic to both the totals and to MPI calculated from the data only in the urban area are shown with high coefficients of 0.990 and 0.980, respectively, as shown in Table I. The fact is very important in consideration of the epidemic. Human patients were usually distributed all over Kyoto City, but in 1966 when a large epidemic occurred, they occurred mainly in the urban area and majority of C. tritaeniorhynchus were caught in the urban area; 588 at station D, 338 at E, 354 at H and 198 at G on July 25, and 435 at D on August 16. Such large numbers of mosquitoes collected in the urban area have never been seen after that year. It is considered that virus transmission resulted from bites of mosquitoes immigrated from the suburban area where they were infected with the virus.

Makiya (1975) proposed the use of Williams’ mean obtained by log (x+1) transformation of the data of serial mosquito collections with a light trap. This transformation is effective to normalized the variation between the mosquito numbers, and may be useful to show population abundance from the data on number of mosquitoes collected at stations sampled at random. On the basis of the data of mosquito collections at several fixed stations, among which mosquito numbers are correlated one another in each collection, it is more correct to compare the annual abundance in MPI as shown in Fig. 4. Numbers of human JE patients did not show such high correlation to Williams’ mean as that to MPI, as shown in Table I, although this difference may be insignificant.

Inatomi and his contributors** have carried out mosquito surveys with light traps in a high epidemic area of JE around Kurashiki City, Okayama Prefecture, every year starting in 1950. These data are valuable, but not used for further analysis of the epidemic of JE. In these data, however, remarkable decrease of C. tritaeniorhynchus has been found in recent years and the tendency of the decrease in number of this species is likely to be the case not only in Kyoto City and Okayama Prefecture, but all over Japan. It may be correct that this decrease is an important factor in the reduction of human JE patients in recent years. For these reasons it was explained by Shimada (1974) that reduction of BHC dusting to rice fields has caused increase in natural enemies of mosquito, or by Kamimura and Watanabe (1972) that the intermittent irrigation or the early planting of rice plants made unsuitable conditions for breeding

** The annual results of mosquito collections at some places in Okayama Prefecture have been successively published mainly in Okayama Igakkai Zasshi from the first paper by Inotomi and Kimura (1955) till the recent one by Inatomi et al. (1974).
mosquito larvae. Of course, the recent decrease of the vector mosquito in Japan may correlate with a number of factors including unknown ones. The decrease of vector mosquitoes, having been noticed as a common phenomenon all over Japan, has probably been caused by some common factors. Those two factors described above do not seem to be such important common factors, because marked reduction of mosquito larvae has recently been seen at rice fields near Kyoto City where BHC dustings or intermittent irrigations have never been operated.

Such herbicides as CNP (MO), p-nitro-phenyl 2,4,6 trichloro-phenyl-p, nitrophenyl ether and Nitrofen (NIP), 2,4-dichlorophenyl-p, nitrophenyl ether have commonly been used for rice plant cultivation in recent years. They are not only effective against mosquito larvae, as shown by Maeda et al. (1976), but may act on the biota of rice field. The use of these herbicides has spread all over Japan in recent years. The outputs (cited from Japan Pesticide Information) of herbicides including those of BHC are shown in Fig. 6; they are inversely correlated with the incidence of JE in Japan. Oya (1970) described in his review that remarkable reduction of patients was found in Japan and Korea but not in Formosa. It is interesting that his findings coincided with the fact a large amount of herbicides has been exported from Japan to Korea but not to Formosa, and that these herbicides can not be used on rice fields in subtropic or tropic regions, because these are ineffective on weeds in a high temperature climate. From the above fact, use of the two herbicides may be another important factor in the reduction of the vector mosquitoes.
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REFERENCES


