Ecological studies on Japanese encephalitis (JE) in Osaka Prefecture

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**Abstract:** Long-term field studies on Japanese encephalitis (JE) in Osaka Prefecture revealed the following points. 1) Though there were 71–346 human cases in the epidemic years of 1965–1967, the incidence of JE has markedly decreased since 1968. In addition, there has been a marked decrease in the number of districts with developed paddy fields and high density of swine farms since 1965. 2) The abundance of *Culex tritaeniorhynchus* decreased annually during the years 1968–1997. 3) In the 1990s the mosquitoes collected from many piggeries were not infected with the JE virus. In most of the districts, the mosquito populations may be far below the critical level for JE virus infection. 4) It was noted that the critical level of mosquito populations below which the JE virus could no longer be isolated varied according to such environmental conditions as the scarcity of paddy fields near a piggery and the breeding number of swine per piggery. As large-scale breeding farms having a large number of swine per piggery have recently been increasing, a rise in this critical level was observed. 5) In 1–4), from the latter half of 1960s to the 1990s, the total absolute amount of infected mosquitoes in Osaka Prefecture markedly diminished, greatly reducing the possibility of JE virus transmission to humans. 6) Further, it seemed probable that according to the small number of swine per piggery, high enough density of swine farms might be necessary for producing a large absolute amount of infected mosquitoes, which in turn brought about the outbreaks of JE, in the 1960s because small-scale breeding farms were common. Thus, it seemed that the environmental condition for mosquito breeding such as the density of swine farms is one of the essential factors involving the epidemics of JE.

**INTRODUCTION**

In Osaka Prefecture, there were 346 human Japanese encephalitis (JE) cases in 1966; then the number of patients decreased yearly and low prevalence has been continuing in recent years. Besides, there were dramatic decreases in the area of paddy fields and in the number of swine farms since 1965 (Nakamura et al., 1992).

By field investigations (Nakamura et al., 1999b), it was noted that three types of areas were recognized according to the abundance of *Culex tritaeniorhynchus* and results of JE virus isolation from mosquitoes, depending upon the suitability of environmental conditions for the mosquito breeding in the terrain where mosquitoes were collected.
Therefore, we investigated in a surveillance performed between 1968 and 1997 whether the number of *Cx. tritaeniorhynchus* collected tended to decrease annually in each district in Osaka Prefecture, and whether JE virus isolation showed a constant yearly tendency, for example, whether there was an increase of districts where the JE virus was not isolated from mosquitoes.

**INVESTIGATION METHODS**

**Number of patients**

The number of patients was obtained from the Annual Reports of Infectious Disease Surveillance Group of Osaka, "Japanese encephalitis and unknown viral diseases in Osaka Prefecture", I-XXXIII (1966–1998).

**Paddy field area and the number of swine-breeding farms**

The paddy field areas and the number of swine-breeding farms (piggeries) were obtained from the statistical report of Osaka Prefecture Agricultural Census (Statistical Information Division, Ministry of Agriculture, Forestry, and Fisheries). To investigate yearly changes in paddy fields and piggeries in each district, the paddy field area planted was presented as the rate of paddy field area to all areas and the number of piggeries was presented as the density per 1 km².

**Collection of mosquitoes**

Mosquitoes were collected once a week between sunset and sunrise using a light trap placed in cowsheds and piggeries. The light trap was set at the same place every year.

*Culex tritaeniorhynchus* for virus isolation were collected near the barn by one person for one hour after sunset using a dry ice-light trap and an insect net. In 1968 and 1969, mosquitoes were collected using an insect net and insect-aspirating tubes. Further details were described in our previous study (Nakamura et al., 1999).

b). The number of sites for mosquito collection was 3 to 6 in a year.

The mean number of mosquitoes collected in July and August was calculated by dividing the total number of mosquitoes collected by frequency of collection. The number of swine was fewer than that in average years in 1992–1994 and 1997 in Mihara, and 1988 and 1989 in Ibaraki. According to Gillies and Wilkes (1969, 1970), the distance at which *Cx. tritaeniorhynchus* is attracted to two calves in the field was almost twice that for attraction to one calf. Therefore, to compare the numbers of mosquitoes collected in all years, the number of swine in an average year (mean number) was divided weekly by the number of swine in that week, and the value was multiplied by the number of mosquitoes actually collected to obtain an estimate. Then, the mean number of mosquitoes collected was calculated using this estimate.

In addition, after 1983 in Sijyonawate, as the number of milking cows raised was about one and a half times more than that before 1982, the number of mosquitoes collected was multiplied by two-thirds.

**Virus isolation**

In principle, 100 females of *Cx. tritaeniorhynchus* which had not sucked blood were handled as one pool, and 10 pools of mosquitoes per one collection site were used weekly for JE virus isolation. Further details were described in our previous study (Nakamura et al., 1999b).

The minimum infection rate (MIR) was calculated by dividing the number of positive pools by the number of mosquitoes tested, and multiplying by 1,000, that is, the value per 1,000 mosquitoes tested. Since the period of virus isolation differed in some years, the period was limited to July and August for the minimum infection rate and for the number of collection sites in which the JE virus was not isolated from mosquitoes.

All results of mosquito collection and virus isolation from mosquitoes were re-

RESULTS

Yearly changes in the number of JE patients
In Osaka Prefecture, there were 71–346 human JE cases between 1965 and 1967, then the number decreased rapidly. During 1968–1972, 6–20 human cases occurred every year, but thereafter, the numbers of patients were only 0–3. After 1987, one case occurred in 1987 and in 1990, and there has been no patient reported since 1991.

Decreases in paddy field area and the number of swine-breeding farms
Yearly changes in the relationship between the rate of paddy field area and the density of piggeries are shown in Fig. 1. The values in 1965 and 1975, which were previously reported (Nakamura et al., 1994), are shown in the left figure, and additionally those in 1985 and 1995 in the right figure. In 1995, because the rate of paddy field area and density of piggeries were almost the same in many districts, quite a number of districts were omitted.

There was no tendency of positive correlation between the paddy field area and the number of piggeries. However, as districts in which paddy fields (larval breeding place of Cx. tritaeniorhynchus) and swine (an amplifier of JE virus) coexist, the number of districts with a rate of paddy field area of 15.0% or higher and a density of piggeries of 0.2 or higher was 9 out of 44 districts in 1965, but it decreased to 1 in 1975, then to 0 in 1985. The number of districts with 10.0% or higher rate of paddy field area and 0.1 or higher density of piggeries was 19 in 1965, and it decreased to 3 in 1975, 2 in 1985, then to 0 in 1990 (not included in Fig. 1). In Osaka Prefecture, the number of districts with a high rate of paddy field area and quite a high density of piggeries sharply decreased between 1965 and 1975. In addition, during the years 1965–1995 the number of districts without piggeries greatly increased from 5 to 39, progressing the
Fig. 2. Yearly fluctuations in the abundance* of *Culex tritaeniorhynchus* females collected at barns in different parts of Osaka Prefecture, 1968–1997.

* The mean number of mosquitoes per night in light traps collected weekly in July and August.

Localization of piggeries extremely.

**Yearly changes in number of *Cx. tritaeniorhynchus* collected**

For the barns in which mosquito collection has been continued for a relatively long time, yearly changes in the mean number of mosquitoes collected are shown in Fig. 2.

There were no barns in which mosquito collection was continued in all years from 1968 to 1997. Though the period was 10 years or shorter in many barns, the points to be noted are as follows.

1) In all Ibaraki, Sijyonawate, Izumi and Matsubara districts, the number of *Cx. tritaeniorhynchus* tended to decrease between 1968 and 1974.

2) In Osaka in Sijyonawate City (Osaka), Minoo, Mihara, Kumatorl, and Kaminogomoyama in Izumisano City (Moyama), mosquitoes were collected until 1997, but the first year of mosquito collection varied among the districts. However, in all these collection sites, the number of mosquitoes collected tended to decrease yearly in spite of regional differences.

3) In Ibaraki, where mosquitoes were collected between 1968 and 1989, the mean number of mosquitoes collected was quite large (0.5×10^4 or more) in 1969 and 1979. But the numbers were not above 0.5×10^4 in 10 years after 1980. Therefore, the number of mosquitoes collected decreased yearly in Ibaraki.

4) In Sijyonawate where mosquitoes were collected between 1970 and 1991 (the distance between this barn and the one in Osaka in the same City was about 3 km), the overall number collected was the largest in 1982–1984, and there was no tendency of decrease in the years between 1970 and 1981. However, after 1982, the number tended to decrease yearly.

Based on these findings, except for Sijyonawate, the abundance of *Cx. tritaeniorhynchus* tended to decrease yearly in
Table 1. JE virus isolations from unfed Culex tritaeniorhynchus females collected at weekly intervals during July and August at barns in Osaka Prefecture, 1968–1997.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of collection sites</th>
<th>No. of pools</th>
<th>No. of mosquitoes tested</th>
<th>MIR$^a$</th>
<th>Date of JE virus isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Tested</td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1968</td>
<td>4</td>
<td>1 (0)</td>
<td>118</td>
<td>8</td>
<td>10,801</td>
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<td>3</td>
<td>0</td>
<td>203</td>
<td>8</td>
<td>18,842</td>
</tr>
<tr>
<td>1970</td>
<td>6</td>
<td>2 (1)</td>
<td>274</td>
<td>26</td>
<td>25,132</td>
</tr>
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<td>6</td>
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<td>12</td>
<td>23,810</td>
</tr>
<tr>
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<td>4</td>
<td>0</td>
<td>175</td>
<td>20</td>
<td>15,425</td>
</tr>
<tr>
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<td>4</td>
<td>0</td>
<td>176</td>
<td>22</td>
<td>15,731</td>
</tr>
<tr>
<td>1974</td>
<td>5</td>
<td>5 (3)$^b$</td>
<td>97</td>
<td>0</td>
<td>6,985</td>
</tr>
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<td>4</td>
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<td>6</td>
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<tr>
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<td>3 (2)$^c$</td>
<td>179</td>
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<tr>
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<td>217</td>
<td>36</td>
<td>20,622</td>
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<tr>
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<td>190</td>
<td>26</td>
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<tr>
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<td>5</td>
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<td>329</td>
<td>53</td>
<td>32,204</td>
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<tr>
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<tr>
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<tr>
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<td>4</td>
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<td>145</td>
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<td>12,740</td>
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<tr>
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<td>1</td>
<td>12,334</td>
</tr>
<tr>
<td>1997</td>
<td>4</td>
<td>4 (3)</td>
<td>64</td>
<td>0</td>
<td>4,683</td>
</tr>
</tbody>
</table>

$^a$ Minimum infection rate/1,000. $^b$ JE virus was isolated from 1 pool of Cx. tritaeniorhynchus collected at one cowshed and two piggeries respectively, in September. $^c$ JE virus was isolated from total 12 pools of Cx. tritaeniorhynchus collected at three barns in September. $^d$ One of these cowsheds was at the side of piggery.


Results of JE virus isolation from mosquitoes
The results of JE virus isolation from mosquitoes collected in 3–6 cowsheds and piggeries in 1968–1997 are summarized in Table 1.

The minimum infection rate varied in a range from 0.000 in 1974, 1995, and 1997 to 2.871 in 1987. After 1993, the minimum infection rate was low for five years, only 0.000–0.354. The earliest date of JE virus isolation from mosquitoes varied from July 5 in 1982 to September 9 in 1974. The latest JE virus isolation varied from August 15 in 1969 to September 16 in 1976, 1980, 1981, and 1987, excluding 1974 and 1996 in which the JE virus was isolated only once. However, there was no constant yearly tendency in these changes. The JE virus was not isolated from at least one cowshed or piggery every year after 1988. The JE virus was not isolated from one piggery in 1993 and 1994, and from all three piggeries between...

If JE virus isolation rate changes by the number of *Culex tritaeniorhynchus* collected as in the combination of *Culex tarsalis* and St. Louis encephalitis virus (Reeves, 1968, 1971; Olson et al., 1979), the minimum infection rate can be compared only when the numbers of mosquitoes collected are similar. Therefore, we investigated whether a negative correlation is observed between the numbers of mosquitoes collected and minimum infection rates, and whether the threshold of the number of mosquitoes collected below which the JE virus is no longer isolated from mosquitoes is observed in the following three types of areas. a) In large paddy-field areas with two or more piggeries, a transmission cycle of the JE virus (mosquitoes → swine → mosquitoes) was often established at an early stage in the epidemic period and every year a large population of *Culex tritaeniorhynchus* appeared, increasing the density of infected mosquitoes. b) In large paddy-field areas with a single piggery, the mosquito populations were small, the transmission cycle of the JE virus did not often occur, and the density of infected mosquitoes was low. c) In large paddy-field areas with a single piggery, the abundance of mosquito populations was smaller than that in b). In the c) areas, one or two of the following conditions were lacking: 1) the paddy field area around the piggery is large, 2) the number of swine raised is at least 1,000, and 3) the paddy field is located very close to the piggery (Nakamura et al., 1999b). In the case of a) and b), as previously reported in detail (Nakamura et al., 1999b), the relationship between the mean number of mosquitoes collected and minimum infection rate in Hineno and in Moyama is shown in Fig. 3. The surveillance was performed only in 1970 and 1971 in Minaminakaokamoto (Okamoto) (the number of swine, 1,000) in Izumisano City (Fujito et al., 1971, 1972), which are additionally presented in Fig. 3. Hineno was classed as a) because of large paddy-field areas with two or more piggeries. In Moyama, the nearby piggery was moved to another prefecture in 1992, and only one piggery remained (1,000). Therefore, after 1992, Moyama was classed as b) as well as Okamoto, which met all the above-mentioned conditions of 1)–3). In these three piggeries, large paddy fields were located nearby throughout the
period of mosquito collection.

Although there was considerable variation in the relations, the value varied depending on the presence or absence of a nearby piggery. In the case of a), Hineno and Moyama (1987-1991), where a nearby piggery was present, the mean number of mosquitoes collected was approximately 2.1-18.8×10^4 and the minimum infection rate was 1.416-3.468. In contrast, after the nearby piggery was removed in Moyama in 1992, the mean number of mosquitoes collected was approximately 0.12-3.0×10^4 and the minimum infection rate was 0.000-1.737. When the mean number of mosquitoes decreased to 1.0×10^4 or below, the JE virus was not isolated.

The correlation coefficient between the mean number of mosquitoes and minimum infection rate was calculated until 1991, but no relationship was observed (r = -0.409, P > 0.05).

The correlation coefficient between the mean number of mosquitoes and minimum infection rate was also investigated including the values in 1992-1994 in Moyama, and 1970 and 1971 in Okamoto, but no relationship was observed (r = -0.668, P > 0.05).

In the case of c) in which 1 or 2 of the above-mentioned conditions were lacking, the relationship between the mean number of mosquitoes collected and minimum infection rate is shown in Figs. 4 and 5. In
Mihara (the number of swine, 60-80), Higashiosaka (100-150), and Osaka (several thousands), the paddy fields were located more or less close to the piggeries at least at the beginning of mosquito collection. In Mihara, the value of 1985 is presented in Fig. 2, but not in the upper figure in Fig. 4, because mosquitoes for virus isolation were not collected in that year. In 1984 swine were vaccinated, and in 1992 and 1993 only seropositive swine were raised. Therefore, these three years were also excluded.

In Mihara, negative correlation was observed between the mean number of mosquitoes and minimum infection rate, excepting the years with the minimum infection rate of 0.000 (r=−0.816, P<0.05). Among the cases in which the JE virus was not isolated from mosquitoes, the largest mean number of mosquitoes collected was 1,434 in 1991. In contrast, among the cases in which the JE virus was isolated, the smallest mean number of mosquitoes collected was 563 in 1994, showing that the former was larger than the latter. The number of mosquitoes tested were 1,157 and 216 in the former and latter, respectively. In 1989, 1995–1997 in which the mean number of mosquitoes collected was 400 or less, the JE virus was not isolated from mosquitoes.

In Higashiosaka, as shown in the middle figure in Fig. 4, the yearly fluctuation in the mean number of mosquitoes collected was only 300–1,500, showing no relationship between the mean number of mosquitoes and minimum infection rate (r=−0.156, P>0.05). The mean numbers of mosquitoes collected in two years in which the JE virus was not isolated were 395 in 1974 and 462 in 1976, but the JE virus was isolated in 1977 in which the mean number of mosquitoes collected was the smallest; 303. However, the numbers of mosquitoes tested in years in which the JE virus was not isolated were 1,193 and 1,343, while those in years in which the JE virus was isolated were 3,065–7,961 (mean: 5,780.5), showing obviously larger numbers.

In Osaka, where there was only a terraced paddy field descending a small ravine, the mean number of mosquitoes collected was larger than 5,000 only in one year during the eight-year surveillance period, and the JE virus was isolated from mosquitoes in only two years (Fig. 4, bottom).

The mean numbers of mosquitoes collected (number of mosquitoes tested) in two years in which the JE virus was isolated were 5,115 (5,482) in 1990 and 79 (961) in 1992, and the mean numbers of mosquitoes collected (number of mosquitoes tested) in six years in which the JE virus was not isolated were 17 (323)–2,323 (2,315).

In Mihara, Higashiosaka, and Osaka around which there are no large areas of paddy fields, the largest mean numbers of mosquitoes collected when the JE virus was not isolated from mosquitoes were 462, 1,434 and 2,323, respectively, though the difference in the numbers of swine raised between the first two piggeries and the last one was large.

In Fig. 5, the relationship between the mean number of mosquitoes and minimum infection rate in piggeries in Ibaraki (the number of swine, 30–40), Kozu (200–250) and Higashikuraji (several thousands) in Katano City (mosquitoes were collected in only two years) are shown, where there were no paddy fields near the piggery from the beginning. In Ibaraki, mosquitoes were also collected in 1988 and 1989 (Fig. 2), but piglets were not raised in 1988 and the breeding of piglets started on August 14, 1989. Therefore, these two years were excluded. Swine were vaccinated between 1968 and 1970. In 1972, 1974, and 1976, 4–8 sentinel swine were kept, but the other swine were vaccinated. In these years, the number of JE virus-sensitive swine may have been lower than that in an average year. Therefore, we investigated the negative correlation between the mean number of mosquitoes and minimum infection rate in 1971, 1973, 1975, and 1977–1987.
In the case of Ibaraki, in which the distance between the piggery and paddy field was about 200–300 m, and the number of swine raised was the smallest; 30–40, the values varied largely, showing no relationship between the mean number of mosquitoes and minimum infection rate \( (r = 0.189, P > 0.05) \). The mean number of mosquitoes collected was 876 in 1997 in which the JE virus was not isolated, but the JE virus was isolated in three years in which the mean number of mosquito collected was 430–761. A large number of mosquitoes (7,550) was tested in 1981 in which the mean number of mosquitoes collected was 430. The number of mosquitoes tested in years in which the JE virus was isolated was 2,988–8,496 (mean: 6,036.1), but the tested number was small (1,475) in a year in which the JE virus was not isolated.

Regarding Kozu and Higashikuraji where the mean number of mosquitoes collected was almost the same in spite of great difference in the numbers of swine raised between the two piggeries, the JE virus was quite frequently isolated in Kozu where the distance between the piggery and paddy field was only about 100 m, but the virus was not isolated from Higashikuraji where the distance between the piggery and paddy field was 500–600 m (there was a small paddy field at about 200 m from the piggery), although 4,065 and 2,733 mosquitoes were tested in the two years and the mean numbers of mosquitoes collected were quite large: 6,498 and 9,755, respectively, despite the fact that several thousands of swine were maintained as in Osaka. In four piggeries other than those in Kozu and Higashikuraji, the largest mean number of mosquitoes collected among the cases in which the JE virus was not isolated was larger than the smallest mean number among the cases in which the JE virus was isolated. However, in Mihara and Osaka, the JE virus was not isolated in four out of five years and in five out of six years in which the mean number of mosquitoes collected was smaller than the largest number among the cases without the JE virus isolation. In Ibaraki and Higashiosaka, since the abundance of mosquitoes did not decrease compared to those in Mihara and Osaka, analysis like that described above could not be performed.

It was noted that i) in the case of b), the threshold of the number of mosquitoes collected below which the JE virus is no longer isolated from mosquitoes in Moyama, 10,000 was quite higher than the largest mean number of mosquitoes collected of 462–2,323 among the cases without JE virus isolation in Mihara, Higashiosaka, Osaka, Ibaraki in the case of c), and ii) such threshold was not constant, and it may vary depending upon the above-mentioned conditions of 1)–3).

**DISCUSSION**

In Osaka Prefecture, the abundance of *Cx. tritaeniorhynchus* decreased yearly from 1968 to 1997, excluding Sijyona-
wate, as in Kyoto City (Maeda et al., 1978). As an exception, the number of mosquitoes collected was the largest in 1982–1984 during the total surveillance period in Sijyonawate. However, in Ibaraki and Higashiosaka, the number of mosquitoes collected did not increase in these years. Though in 1980 and 1985, there were no appreciable differences in the rate of paddy field area among these three districts, the density of barns, that is the number of farms breeding milking cows, beef cattle and swine per 1 km², was obviously higher in Sijyonawate (0.635–0.529) than in Higashiosaka (0.308–0.113), apart from the smallest density in Ibaraki (0.067–0.027). However, in Sijyonawate from 1985 to 1990 the density of barns decreased by half, bringing about unfavorable environmental conditions for mosquito breeding for the other districts as well. As a result, after 1982 the number of mosquitoes collected tended to decrease yearly irrespective of the favorable meteorological conditions for mosquito breeding as in Ibaraki (Nakamura et al., 1999a). In Osaka Prefecture, Cx. tritaeniorhynchus acquired resistance to agricultural insecticides around 1980, as in other prefectures (Kamimura and Maruyama, 1983; Yasutomi and Takahashi, 1987; Yoshida et al., 1984; Nakamura et al., 1984). Since regional differences in acquired resistance were not observed in Osaka Prefecture (Yoshida et al., 1984; Nakamura et al., 1984), an increase in Cx. tritaeniorhynchus populations due to insecticide-resistance may not have occurred in Ibaraki and Higashiosaka as in Toyama Prefecture (Watanabe et al., 1990) because of unfavorable environmental conditions for mosquito breeding, comparing with Sijyonawate in which it was as before 1985.

Acquisition of insecticide-resistance in Cx. tritaeniorhynchus may be counter evidence to growth inhibition of Cx. tritaeniorhynchus populations by agricultural chemicals (Shimada, 1974; Buei et al., 1977; Maeda et al., 1978; Mogi, 1978). However, in 1980, the environmental conditions in Osaka Prefecture were comparable to or more suitable than those in Sijyonawate, such as 6.0% or higher rate of paddy field area and 0.6 or higher density of barns, only in seven (no piggery present in one district) out of 44 districts. Probably, the environmental changes in the whole area in Osaka Prefecture are the reason for the lowered incidence to 0–3 patients in the 1980s despite the fact that Cx. tritaeniorhynchus acquired insecticide-resistance.

In the 1990s, the number of piggeries in which the JE virus was not isolated from mosquitoes tended to increase yearly, which may have been due to an increase in the number of districts in which the abundance of mosquitoes decreased to below the critical level of JE virus isolation. However, such threshold was not constant, and there was considerable variation in the threshold in each district in which mosquitoes were collected.

Furthermore, although cases of JE virus isolation may increase when the number of mosquitoes tested is larger than a certain level, one reason for the absence of a clear threshold may have been the fact that the mean number of mosquitoes collected and the number of mosquitoes tested were not directly proportional. Moreover, the JE virus was isolated in Mihara and Osaka despite the fact that the number of mosquitoes tested was small (216–961). When the abundance of mosquitoes is small and the absolute number of infected mosquitoes is also small, there may be room for accidents occurring in collecting infected mosquitoes. Considering the small mosquito population, the high rate of parous mosquitoes may be responsible for generating room for such accidents.

Infected mosquitoes may be parous, and negative correlation has been observed between the number of mosquitoes collected and the rate of parous mosquitoes. For example, when the numbers of mosquitoes collected were 1,000 and 10,000, the rates of parous mosquitoes were about 50.0% and 15.0–25.0%, respectively (Buei et al.,
1974, 1982).

Why was the critical level of mosquito abundance below which JE virus isolation disappears high in Moyama? In 1995 and 1996 in which the number of mosquitoes collected was below the threshold of JE virus isolation, 2-ME-sensitive antibody was detected in swine on August 3 and July 31, respectively, but an HI antibody positive rate did not reach 100.0% during August (Kimura et al., 1996, 1997).

In Okamoto where about 1,000 swine were raised as in Moyama, all eight sentinel swine became HI antibody-positive at the end of August in 1971 in which the mean number of mosquitoes collected was $4.05 \times 10^4$. However, in 1970 in which the mean number of mosquitoes collected was smaller ($2.34 \times 10^3$), 10 sentinel swine were not converted to HI antibody-positive by the end of August (according to the reports VI (1971) and VII (1972) of Infectious Disease Surveillance Group of Osaka).

The number of swine developing viremia at one time is unknown. However, when about 1,000 swine are maintained, the number of mosquitoes that sucked blood per one swine decreased as the abundance of Cx. tritaeniorhynchus decreased, and thus, the cases of including infected mosquitoes in blood-sucking mosquitoes may have decreased. Accordingly, production of infected mosquitoes in the whole piggery may have decreased. Therefore, although the abundance of mosquitoes was still quite large, a threshold of the number of mosquitoes collected below which the JE virus is not isolated was observed.

In Mihara, Higashiosaka, Osaka, and Ibaraki, the JE virus was always isolated when the number of mosquitoes tested was 3,000 or more. In Moyama, large numbers of mosquitoes, 7,880 and 6,869, were tested in 1995 and 1996, respectively, but the JE virus was not isolated from mosquitoes, which may have been due to the following: 1) the absolute number of infected mosquitoes was small, and 2) infected mosquitoes were not collected because of the low rate of parous mosquitoes.

The negative correlation between the number of mosquitoes collected and minimum infection rate was observed only in Mihara. Since negative correlation has been observed between the number of Cx. tritaeniorhynchus collected and the rate of parous mosquitoes (Buei et al., 1974, 1982), a similar relationship may basically exist between the number of mosquitoes and the minimum JE virus infection rate. Therefore, it may be difficult to investigate whether the minimum infection rate with the JE virus changed yearly, to which we did not refer.

It was considered that the threshold is not constant but varies depending upon the suitability of the environmental conditions, such as an absence of paddy fields near the piggery and how many of the above-mentioned conditions are met. This is equally true of the abundance in mosquito populations, and of the production of infected mosquitoes (Nakamura et al., 1999b). Further, under the suitable environmental conditions, such as those in Hineno where the transmission cycle was established every year, ensuring effective repeating of blood-feeding, oviposition, and re-feeding, in large paddy-fields with four piggeries (total about 2,120 swine), the threshold in itself does not exist, showing the highest mean minimum infection rate in our study, though the total number of swine raised at four piggeries was obviously smaller than that at one piggery in Osaka and in Higashikuraji (Nakamura et al., 1999b). Therefore, it seemed probable that according to the small number of swine per piggery, high enough density of swine farms might be necessary for producing a large absolute amount of infected mosquitoes, which in turn brought about the outbreaks of JE, in the 1960s in which small-scale breeding farms were common (Nakamura, 1988, 1995; Nakamura et al., 1992, 1999b).

In addition, our long-term field studies on JE in Osaka Prefecture revealed the
following points: 1) sharp decrease in the paddy field area and the number of livestock breeding farms was observed in recent years (Nakamura, 1988; Nakamura et al., 1992), 2) yearly decrease in the number of *C. tritaeniorynchus* collected, 3) in the 1990s an increase in the number of districts where the abundance of mosquitoes decreased to the threshold below which the JE virus was no longer isolated, 4) disappearance of districts where the environmental conditions are suitable, such as establishment of the transmission cycle every year without a threshold in itself (Nakamura et al., 1998b), and 5) owing to progressive conversion to large-scale breeding farms in recent years (Nakamura, 1988), an elevation in the threshold of the mosquito abundance level below which the JE virus is not isolated, that is, occurrence of some piggeries in which no JE virus was isolated in spite of quite a large mosquito abundance. Thus, the absolute amount of JE virus-infected mosquitoes produced in the whole area of Osaka Prefecture markedly decreased from the latter half of the 1960s, during which outbreaks occurred to recent years, which may have markedly reduced cases of blood-sucking of humans by JE virus-infected mosquitoes.

In conclusion, we can say that the environmental conditions for mosquito breeding, such as the number of swine raised per piggery and the density of swine farms, is one of the essential factors involved in the epidemics of JE (Nakamura, 1988, 1995; Nakamura et al., 1992, 1999).

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