

## Ecology of Japanese Encephalitis Virus in Japan.

### II. The population of vector mosquitoes and the epidemic of Japanese encephalitis

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**ABSTRACT:** The relation between the population of the vector mosquito, *Culex tritaeniorhynchus*, and the epidemic of Japanese encephalitis (JE) was analyzed with the data obtained in the Nagasaki district during ten years from 1964 to 1973. The results revealed the following points. (1) The starting time of the appearance of overwintered females or newly-emerged males of *tritaeniorhynchus* in each year has no relation to the starting time of JE epidemic as represented by the first isolation of JE virus from *tritaeniorhynchus* or the first appearance of 2-ME sensitive antibody in slaughtered pigs. (2) The start of JE epidemic coincided generally with the rise in the population density of *tritaeniorhynchus*. (3) In the year when the number of JE human cases was large, the accumulated density of *tritaeniorhynchus* throughout the year was generally high. However, only a very few cases appeared in years when the accumulated density of the vector mosquito was lower than a certain critical value which is probably related to the threshold density of vectors for the occurrence of JE epizootic in pigs. (4) It was clearly demonstrated that the number of human cases is directly proportional to the vector density when the pig epizootic is in progress. (5) The infection rate of *tritaeniorhynchus* with JE virus was variable in years, but apparently had no relation to the size of JE epidemic. (6) It was concluded that the possibility for overwintering *tritaeniorhynchus* females to have JE virus in their bodies is, if

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ever, extremely low, because no females just having emerged from hibernation were found parous. (7) The lowering density of *tritaeniorhynchus* in recent years was considered to be one of the most important factors to have induced the reduction of the number of human cases.

Because Japanese encephalitis (JE) is a serious disease in Japan, much literature has been published on the ecology of JE virus. Its important results include that the main vector of JE is *Culex tritaeniorhynchus* and pigs are important in amplifying the virus in nature. However, many aspects of the ecology of JE virus are still incomplete. In the previous paper of this series (Fukumi et al., 1975), two subjects were treated from virological standpoint based on the results obtained from 1964 to 1973 in the Nagasaki district; one was the epidemiology of JE in the epidemic season and the other the possibility of overwintering of the virus in the vector mosquito. The aim of the present paper is to discuss the same subjects from the entomological point of view. Data on mosquitoes have partly been reported particularly for earlier years (Fukumi et al., 1975; Omori et al., 1965; Wada et al., 1967, 1973), however they are differently arranged in the present paper so that mosquito densities can be compared seasonally and yearly.

#### MATERIALS AND METHODS

Mosquitoes were collected by an aspirator at pigsties and cowsheds, and hen houses to a less extent, and also by using a dry ice trap in the field, usually at a regular interval of one week but frequently 2 or more times in a week. Twenty minute catch at animal shelters from one hour after the sunset and one hour catch by a dry ice trap from about sunset were usually made each by one person. However, the starting times of mosquito catches differed slightly in season according to the results on the hourly activity of mosquitoes (Mogi et al., 1970; Wada et al., 1970). All areas where mosquitoes were collected were farm villages near rice fields in various degrees of development, situated around Nagasaki city. Since *C. tritaeniorhynchus* is a dominant mosquito (Wada et al., 1967) and the main vector of JE (Fukumi et al., 1975), other species were not mentioned about in the present paper. As for infections with JE virus in mosquitoes, pigs and humans, the data given in the previous paper (Fukumi et al., 1975) were used.

#### RESULTS

*The appearance of overwintered females and newly-emerged males of tritaeniorhynchus and the epidemic of JE*

Table 1 gives the appearance times of overwintered females and newly-emerged males of *tritaeniorhynchus* and the starting time of JE epidemic. The appearance time of *tritaeniorhynchus* females from hibernation depends on the time of awakening from winter diapause, and varies from year to year probably due to different temperatures in early spring of each

year (Wade et al., 1973). The earliest date for the first appearance of overwintered females was March 13 in 1966 and the latest one was April 8 in 1970.

Table 1. First appearances of overwintered females and newly-emerged males of *Culex tritaeniorhynchus* and first infections with JE virus in mosquitoes and pigs in each year from 1965 to 1973, in Nagasaki prefecture

Year	1965	1966	1967	1968	1969	1970	1971	1972	1973
First appearance of overwintered females*	Mar.31	Mar.13	Mar.27	Apr.2	Mar.26	Apr.8	Mar.29	Apr.4	Mar.27
First appearance of newly-emerged males	May 15	Apr.27	May 11	May14	May 6	May 14	May 11	**	Apr.25
First isolation of JE virus from mosquitoes	May 30	Jun.24	Jun. 23	Jul.22	Aug.1	Jul.19	Jul. 13	Aug.16	Jul.10
First appearance of 2-ME sensitive HI antibody in slaughtered pigs	Jun.22	Jul. 4	Jun.22	Jul.20	Jul.24	Aug.8	Jul. 6	Sep.5	Jul.24

\* Data of only a very few having been collected were ignored.

\*\* Not determined.

The appearance of a new generation of *tritaeniorhynchus* is indicated by males, since only females overwinter. The date in each year for the first appearance of newly-emerged males in Table 1 is based on the collection records of males in swarms near dry ice traps (Kawai et al., 1967). Again, the appearance time of the first males differs greatly in years, depending on the time of oviposition by overwintering females and the condition, particularly the temperature, during immature stages. The earliest date was April 25 in 1973 and the latest one May 15 in 1965.

The starting time of JE epidemic is considered to be represented by the date for the first isolation of JE virus from *tritaeniorhynchus* or for the first appearance of 2-ME sensitive HI antibody in slaughtered pigs. Those dates for each year are given in Table 1 from the previous paper (Fukumi et al., 1975). The time of the first isolation of the virus was early generally in the years when the first 2-ME sensitive HI antibody appeared early, and *vice versa*.

However, as clearly seen in Table 1, there is no relation between starting times of *tritaeniorhynchus* appearance and JE epidemic. This is understandable, because the time of JE epidemic is influenced by the density of vector mosquitoes rather than the starting time of the appearance of overwintered or newly-emerged mosquitoes. The time of JE epidemic will be discussed again in the section of seasonal and yearly changes of *tritaeniorhynchus* density.

#### *Seasonal and yearly changes of tritaeniorhynchus and the number of JE cases*

The population density of *tritaeniorhynchus* in a given area is not determined easily, because the number of collected mosquitoes varies greatly by day and place, as well as by

Table 2. Total numbers of *Culex tritaeniorhynchus* females\*collected by three different methods in the period from early spring to mid-autumn covering completely their active season, in Nagasaki prefecture

Year	Area	Total No. of females		
		by dry ice traps	at pigsties	at cowsheds
1965	Tomachi	16,065.5	1,801.0	2,540.2
	Mogi	14,492.5	888.6	1,553.0
1966	Mogi	5,229.0	1,749.0	1,818.0
	Kaizu	13,804.3	6,486.0	15,320.0
1967	Mogi	2,861.5	795.5	989.0
	Kaizu	6,573.0	4,134.0	10,652.5
1968	Kaizu	1,955.0	733.0	2,529.5
Total		60,980.8	16,586.6	35,402.2

\* Females only when collected by all of the three methods on the same day at each area were totaled; if they were collected by two or more dry ice traps, or at two or more pigsties or cowsheds on a day at an area, their mean was used to get the total.

collection method. Therefore, the population index, by which we can compare population densities with reasonable certainty, was introduced as in the below.

As described earlier, mosquitoes were collected at several areas in the Nagasaki district by using dry ice traps, at pigsties and at cowsheds, usually at one week interval but frequently 2 or more times in a week during the period from early spring to mid-autumn, which covered completely the whole active season of *tritaeniorhynchus*. In Table 2 are given the total numbers, in each year, of females only for the days on which all the three collection methods mentioned above used at an area. Because the times of collections at an area in a year are the same in these three methods, it can be said that mosquitoes were collected most efficiently, on an average, by dry ice traps, next at cowsheds, and least at pigsties.

If we correct the number of collected mosquitoes with the weight which is proportional to the reciprocal of the total number for the four years by each collection method shown in Table 2, then corrected values are comparable one another. Let the totals by dry ice traps, at pigsties, and at cowsheds be a, b, and c, respectively, and the weights to be used for the correction may be given as 1/a, 1/b, and 1/c. However, the weights were modified, for convenience, so that the sum of the weights for the three collection methods was unity. Thus the weight for dry ice trap catches was calculated as

$$\frac{1/a}{1/a + 1/b + 1/c} = \frac{bc}{bc + ca + ab} = 0.1563, \text{ and similarly}$$

$$\frac{ca}{bc + ca + ab} = 0.5745 \text{ for pigsty catches and}$$

$$\frac{ab}{bc + ca + ab} = 0.2692 \text{ for cowshed catches,}$$

where  $a=60,980.8$ ,  $b=16,586.6$ , and  $c=35,402.2$  from Table 2.

As the total of the three weights is one, if the numbers of collected mosquitoes at an area on a given day by the three methods are multiplied by respective weights and

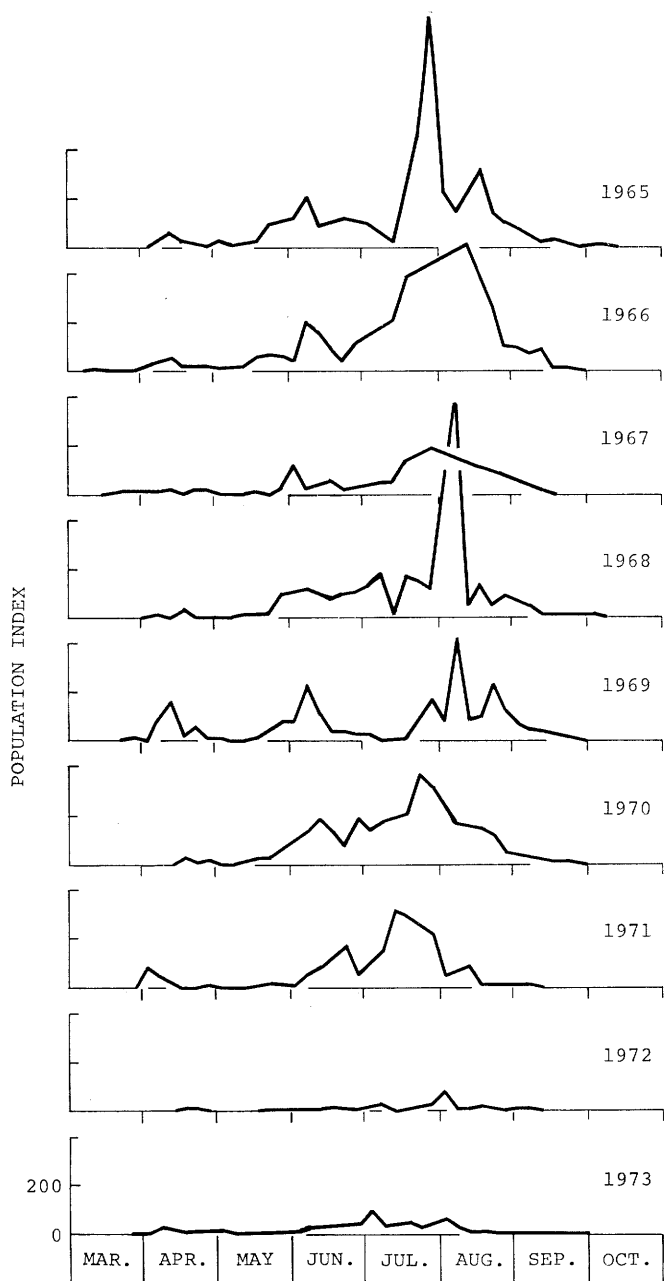


Fig. 1. Seasonal prevalence of population index for five day interval of *Culex tritaeniorhynchus* females at Mogi area from 1965 to 1973.

summed up, then we have the weighted mean of mosquito catches for that day. If two methods only were used to catch mosquitoes, the sum of corrected mosquito catches should be multiplied by 1.5, and if one method only, catches should be multiplied by 3. The value thus obtained may be called the population index.

These indices were then arranged by five day interval for each area, and if there

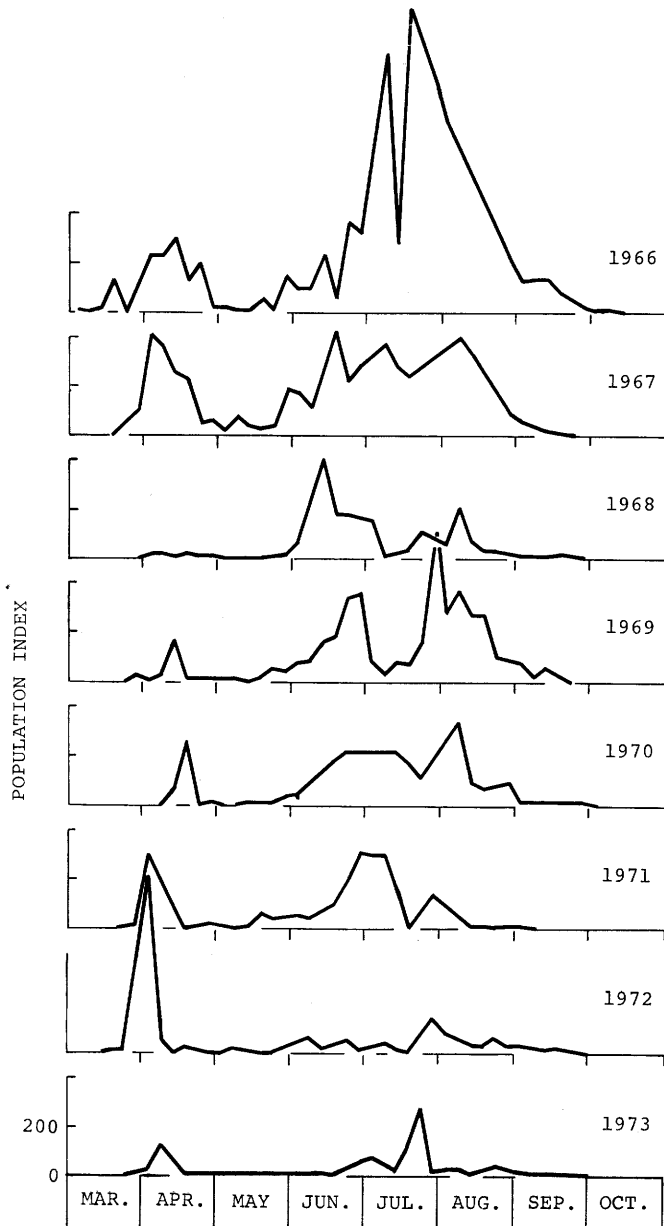


Fig. 2. Seasonal prevalence of population index for five day interval of *Culex tritaeniorhynchus* females at Kaizu area from 1966 to 1973.

are two or more indices in an interval, the average was calculated, and if there is no index, the value was obtained by interpolation. The total of five day interval values throughout a year may be called the population index for a year.

Although mosquitoes were collected at many areas near Nagasaki city, as mentioned earlier, it was at Mogi and Kaizu areas that mosquito catches were made normally at an interval of one week continuously for many years, that is, since 1965 at Mogi and since 1966 at Kaizu. Environmental situations for mosquito ecology, such as rice fields for larval breeding and domestic animals for adult feeding, were remained to be rather unchanged throughout the periods, therefore it is reasonable to compare mosquito prevalences seasonally and yearly in these two areas.

Fig. 1 and 2 show the seasonal changes of the population index (for five day interval) of *tritaeniorhynchus* females from 1965 to 1973 at Mogi area and from 1966 to 1973 at Kaizu area. It is clearly seen in Fig. 1 and 2 that the change in the density of *tritaeniorhynchus* population was remarkable in season at both villages, though the density was generally higher at Kaizu than at Mogi. Females having overwintered abundantly appeared in March and April. The new generation of *tritaeniorhynchus* began to emerge in late April – middle May (see also Table 1), but the density was generally low until late June or early July when rice plants were transplanted in rice fields with water which are the main breeding site for this mosquito. High population density was usually seen in July and August, and after that the number of females active in feeding became very small. Also, the yearly change in density was evident, and generally the population became small in later years, as seen in Fig. 1 and 2, and more clearly in Table 3 which shows the population index for a year each from 1965 to 1973. The factors to induce such seasonal and yearly changes will be reported in a different paper.

To relate the seasonal prevalence of *tritaeniorhynchus* to the time of JE epidemic, the time of the first appearance of 2-ME sensitive HI antibody in slaughtered pigs (from Table 1) was drawn on the prevalence of the mean population index of *tritaeniorhynchus*

Table 3. Population index of *Culex tritaeniorhynchus* for each year from 1965 to 1973 in Mogi and Kaizu areas, Nagasaki prefecture

Year	Mogi	Kaizu	Mean
1965	4,418	7,563*	5,991
1966	5,270	11,585	8,424
1967	1,939	6,957	4,448
1968	3,419	2,200	2,810
1969	2,602	4,180	3,391
1970	3,451	3,456	3,454
1971	2,309	2,766	2,537
1972	404	2,151	1,278
1973	751	1,190	971

\* Mosquitoes were not collected; index was estimated by  $4,418 \times (\text{total of indices for Kaizu from 1966 to 1973}) / (\text{total of indices for Mogi from 1966 to 1973})$ .

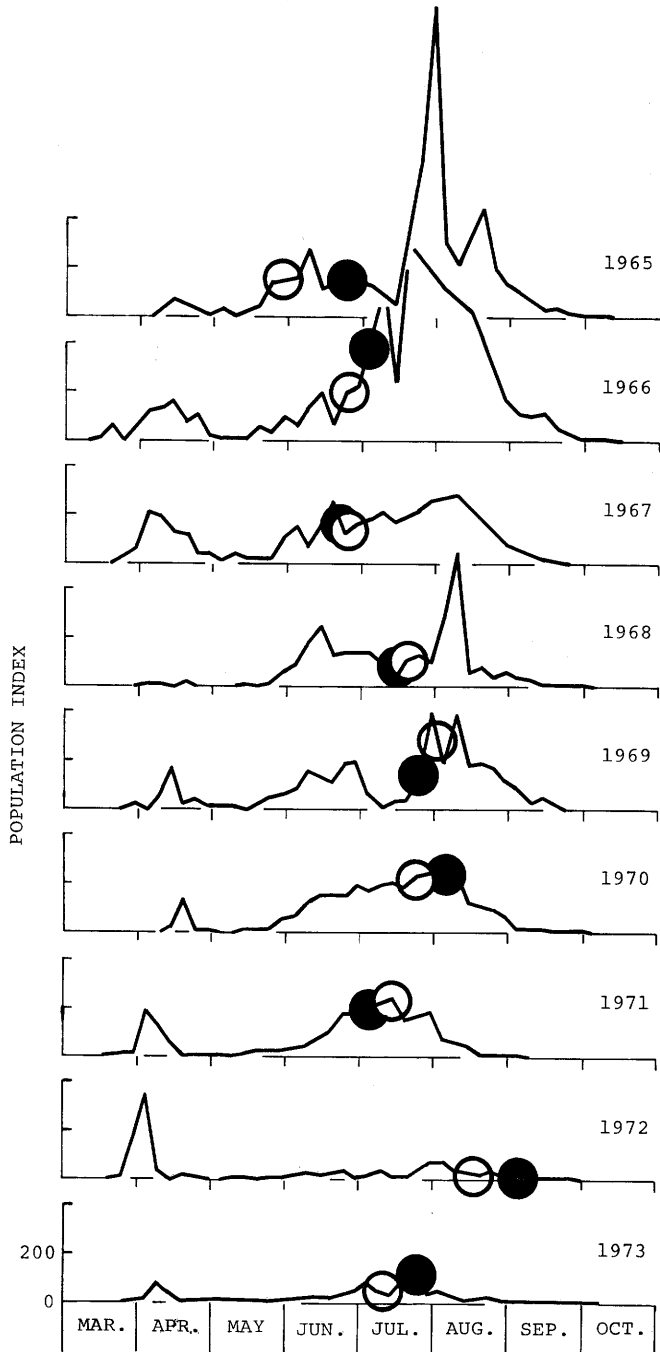


Fig. 3. Starting time of JE epidemic in relation to the seasonal prevalence of *Culex tritaeniorhynchus* females in the Nagasaki district, from 1965 to 1973. The starting time of JE epidemic is represented by the times of the first appearance of 2-ME sensitive HI antibody in slaughtered pigs (black circle) and the first isolation of JE virus from mosquitoes (white circle), and the seasonal prevalence of *tritaeniorhynchus* by the mean population index for five day interval at Mogi and Kaizu (the mean index in 1965, in which mosquitoes were not collected in Kaizu, was obtained proportionally with the index at Mogi in 1965 and the totals of indices throughout the years from 1966 to 1973 at Mogi and Kaizu).



at Mogi and Kaizu in Fig. 3. The start of JE epidemic, as indicated by the first appearance of 2-ME sensitive HI antibody in pigs and the first isolation of JE virus from mosquitoes, coincided generally with the rise in the population density of *tritaeniorhynchus*. In the years from 1965 to 1971, JE epidemic started when the population index of mosquitoes reached approximately 200. However, in 1972 and 1973 the index remained to be small throughout the year, nevertheless JE epidemic occurred. The reason for this may be due to the fact that the mosquito prevalence was based on the data obtained only in two areas. It seems to be probable that the density of *tritaeniorhynchus* was high locally in some other areas than Mogi and Kaizu.

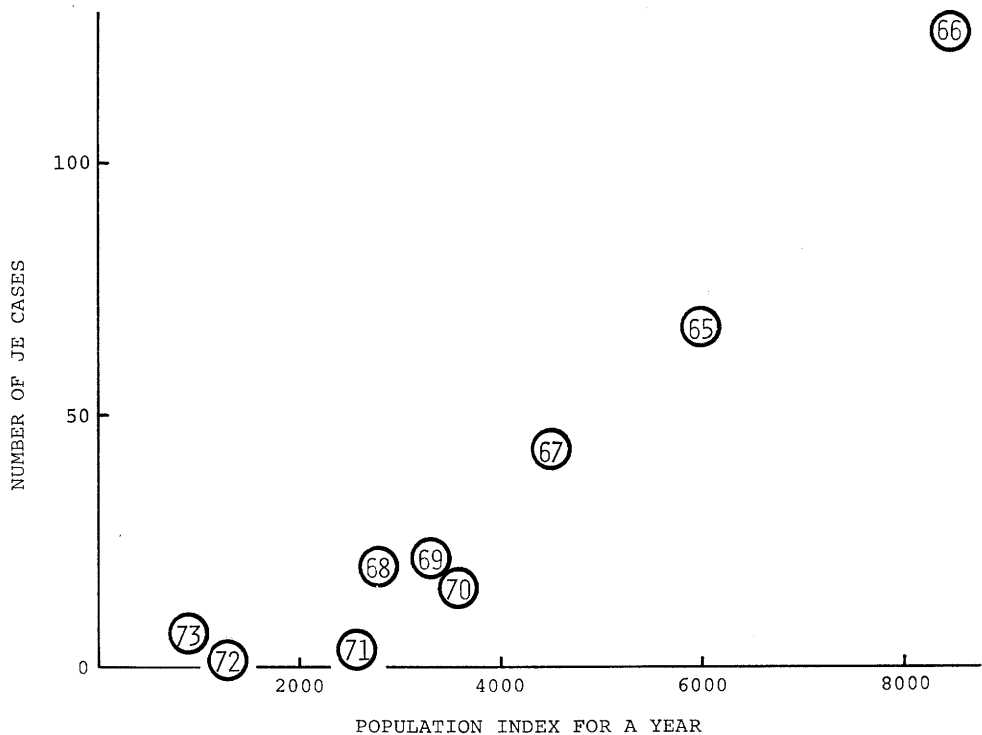


Fig. 4. Relation between the population index of *Culex tritaeniorhynchus* for a year and the number of JE human cases in Nagasaki prefecture from 1965 to 1973.

The relation between the population density of *tritaeniorhynchus* and the size of JE epidemic is more clearly seen than between the density of vectors and the time of JE epidemic. In Fig. 4, the number of JE human cases was plotted against the population index for a year (the total of population indices for five day interval throughout the year) each in the years from 1965 to 1973. The linear relation between them is very apparent in Fig. 4, the higher the density of *tritaeniorhynchus* is, the larger the number of human cases is. Because females of *tritaeniorhynchus* in March and April are those having overwintered and JE virus is not considered to overwinter in mosquitoes (see later section), the relation was illustrated with the total of population indices after May, instead of the

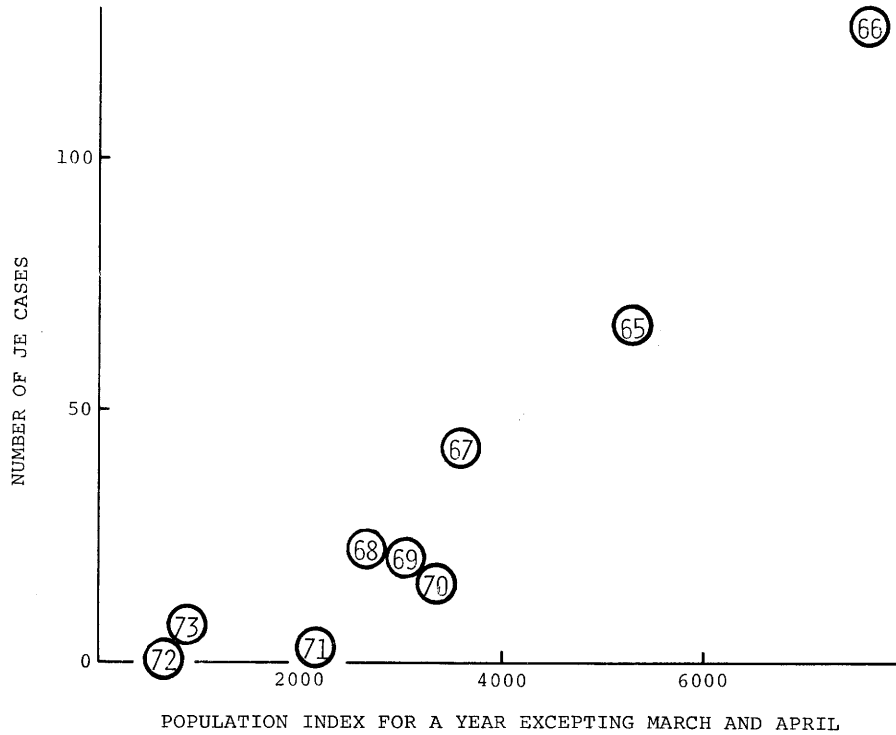


Fig. 5. Relation between the population index of *Culex tritaeniorhynchus* for a year excepting March and April and the number of JE human cases in Nagasaki prefecture from 1965 to 1973.

total throughout the year, in Fig. 5. Again the relation is very close between the two. It is interesting that both in Fig. 4 and 5, when the total of population indices decreased till ca. 2,000, the number of human cases constantly decreased up to nearly zero, and at smaller values of the total of indices than ca. 2,000 the number of cases remained to be extremely small. In other words, only an extremely few JE cases are expected when the total of population indices of *tritaenirhynchus* in a year is less than 2,000, and this critical value is probably related to the threshold density of vector mosquitoes for the occurrence of JE epizootic in pigs (Wada, 1975).

In the above, the relation of the number of human JE cases to the density of *tritaeniorhynchus* in the whole year (Fig. 4) or in the year excepting March and April (Fig. 5) was mentioned. However, the number of human cases is considered to be proportional to the density of *tritaeniorhynchus* when a pig epizootic is in progress. The duration of the epizootic may be given as approximately 40 days, from the data for the rise in HI antibody positive rate in slaughtered pigs, and the starting time of the epizootic may be shown by the time of the first appearance of 2ME-sensitive antibody in pigs. Thus, the number of cases was plotted against the total of *tritaeniorhynchus* population indices in the period of 40 days after the first 2ME-sensitive antibody in each year from 1965 to 1973 in Fig. 6. As expected, the relation between them is directly proportional,

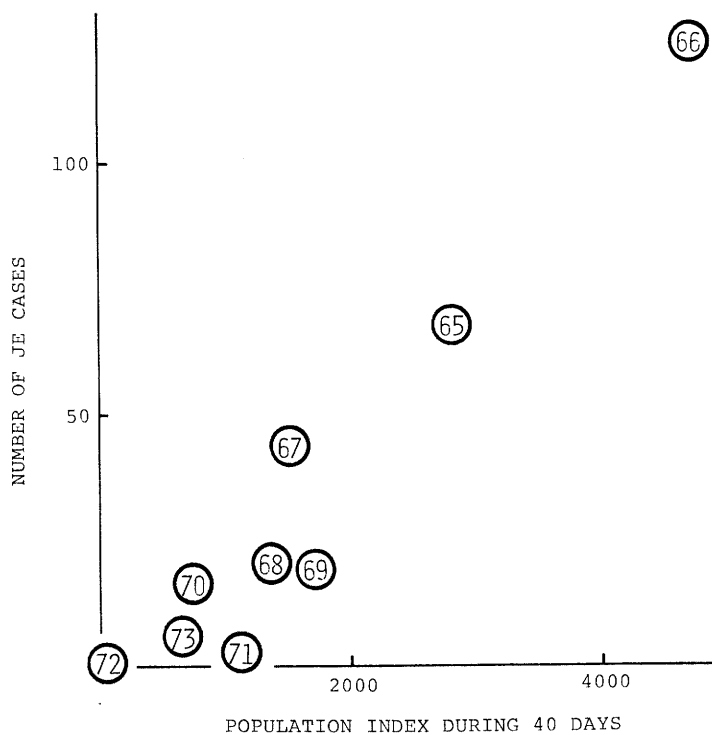


Fig. 6. Relation between the population index of *Culex tritaeniorhynchus* during 40 days after the first appearance of 2ME-sensitive antibody in slaughtered pigs and the number of JE human cases in Nagasaki prefecture from 1965 to 1973.

and there is no such range of *tritaeniorhynchus* density as only an extremely few cases occur as seen in Fig. 4 and 5.

#### *Infection rate of tritaeniorhynchus with JE virus*

Mosquitoes are infected with JE virus normally during the period of the pig epizootic. However, the infection rate of mosquitoes is influenced not only by the number of infected pigs but also by the trend of mosquito density (Wada, 1975). Fig. 7 shows the isolation efficiency in each ten day interval from 1964 to 1973. Here, the isolation efficiency, defined by (the number of JE virus isolates/the number of mosquitoes in inoculated pools) x 1,000, was taken tentatively as a measure of the infection rate, and the cases when the number of mosquitoes was less than 100 were not illustrated for the sake of reliability. It is clear that the isolation efficiency is variable, and there is no relation between the isolation efficiency and the size of JE epidemic. For example, in 1966 the largest number of human cases were reported, however the isolation efficiency was generally lower than other years.

As far as the virus isolation is attempted only from mosquitoes having fed on blood in a pigsty, the infection rate would be approximately proportional to the rate of pigs in

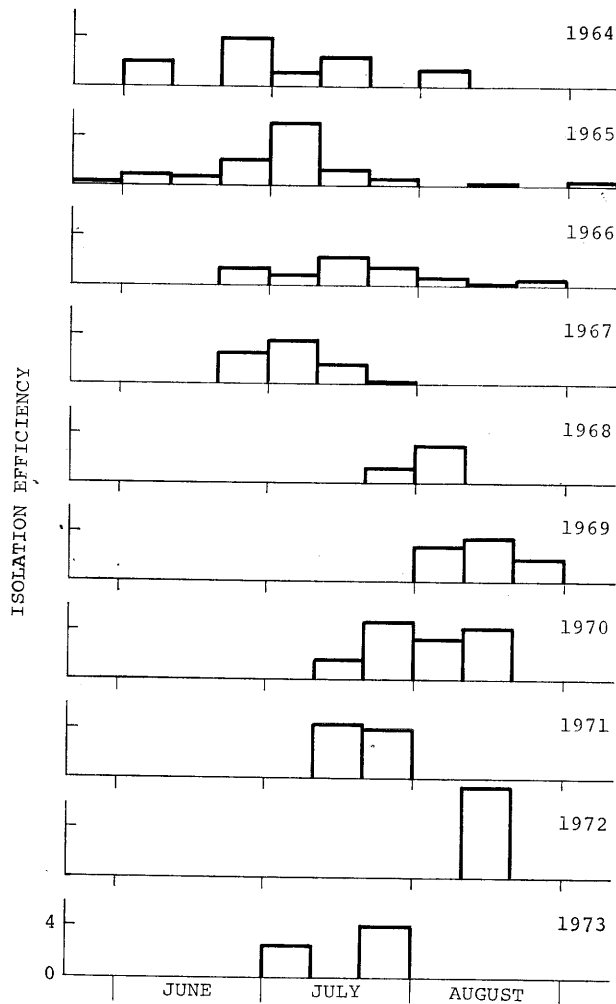


Fig. 7. Isolation efficiency of JE virus in a ten day interval from *Culex tritaeniorhynchus* females in the Nagasaki district from 1964 to 1973. The isolation efficiency was calculated by (the number of JE virus isolates/the number of females in inoculated pools) x 1,000. Isolation efficiencies when the number of mosquitoes was less than 100 were not illustrated for the sake of reliability.

viremia in that pigsty. In this sense, similar infection rates of blood-fed females in pigsties are expected in the years from 1964 to 1973 excepting 1972, because the rate of slaughtered pigs with HI antibody due to JE virus infection reached nearly 100% by the end of summer in every year excepting 1972 in which the rate remained far below 100%. However, mosquitoes, fed as well as unfed, were collected not only at pigsties but also at cowshes and by bry ice traps. Therefore, the isolation efficiency was calculated only with mosquitoes by dry ice traps which did not include individuals fed on any animals, and the results were given Fig. 8.

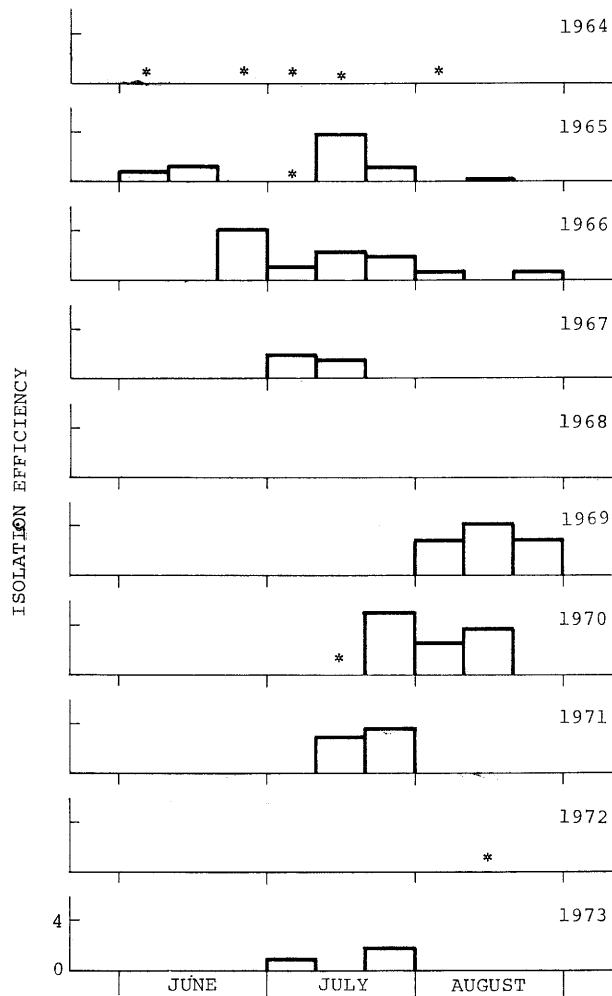


Fig. 8. Isolation efficiency of JE virus in a ten day interval from *Culex tritaeniorhynchus* females collected only by dry ice traps in the Nagasaki district from 1964 to 1973. Asterisks indicate the cases when the number of mosquitoes was less than 100, therefore the isolation efficiency was not calculated, though the efficiency was illustrated in Fig. 7.

If the virus is isolated from mosquitoes collected by dry ice traps, there must be included the mosquitoes which were infected before when they fed on viremic animals. The isolation efficiency of mosquitoes collected only by dry ice traps could not be calculated for some periods because mosquitoes collected by various methods were frequently mixed to make a pool for virus isolation. However, Fig. 8 again shows that there is no relation between the isolation efficiency and the epidemic size.

#### *Age structure of tritaeniorhynchus overwintering population*

Mifune (1965) and Shichijo et al. (1972) demonstrated experimentally that JE virus

can overwinter successfully in infected females of *tritaeniorhynchus*. Many females which had overwintered could be collected in March and April in the Nagasaki district, however no JE virus was detected (Fukumi et al., 1975). One explanation for this may be that *tritaeniorhynchus* females enter hibernation in autumn without feeding on blood, therefore there is no chance for them to be infected. To know whether this explanation is acceptable or not, the females collected in spring and early summer were examined for physiological age by Detinova's method and/or Polovodova's method (Detinova, 1962). The results are given in Table 4.

Table 4. Parous rate of *Culex tritaeniorhynchus* females in spring and early summer in Nagasaki Prefecture

	1970		1973		1974		Total		
	Number examined	Number parous	Number examined	Number parous	Number examined	Number parous	Number examined	Number parous	Percentage parous
March, late	2	0	10	0			12	0	0.0
April, early	19	0	32	1*	10	0	61	1	1.6
middle	1,222	6			120	10	1,342	16	1.2
late	197	43			65	2	262	45	17.2
May, early	1	1			26	17	27	18	66.7
middle					7	3	7	3	42.9
late	101	14			57	21	158	35	22.2

\* A uniparous female retaining a few mature eggs.

Among 12 female mosquitoes collected in late March, none was found parous (with the experience of oviposition). One female (1.6%) of 61 in early April was parous, but it retained a few mature eggs indicating the recent blood feeding after having come out from hibernation. Therefore, those 73 females in total are considered to have entered hibernation in autumn without oviposition. The parous rate of the mosquitoes was still very low (1.2%) in middle April. The rate became high after that and reached 66.7% in early May. This increase in the parous rate is apparently due to the feeding and oviposition in spring. The rate then became lower by the emergence of new nulliparous females.

It is considered from the above that no or negligibly small number of, if any, parous females are included in the overwintering population of *tritaeniorhynchus*. Moreover, the incidence of gonotrophic dissociation (a phenomenon of undevelopment of mature eggs after blood feeding) is rare in overwintering *tritaeniorhynchus* females (Oda & Wada, 1973). Because mosquitoes are infected on feeding on viremic animals, it can be said that the possibility for overwintering *tritaeniorhynchus* to have JE virus is extremely low.

#### DISCUSSION

Takahashi et al. (1973) discussed the problems involved in the quantitative sampling of the vector population of JE virus, and called the attention to the difference in the

number of mosquitoes by method, place, and day. It is important to be kept in mind in attempting the survey of mosquitoes in relation to the epidemiology of JE. The data for *tritaeniorhynchus* used in the present paper were based on the collection by 2 or 3 different methods each at Mogi and Kaizu areas. As far as the *tritaeniorhynchus* population in each area is concerned, the relative density of female mosquitoes was considered to be quite accurate, in view of the very close correlation between densities of adult and larval population. A question remained whether those data obtained in the two areas well represent the real situation of the *tritaeniorhynchus* population in the Nagasaki district. Because there is a great variation in the mosquito density among areas, the data obtained only in two areas may not be sufficient to represent, in some cases, the population in the whole Nagasaki district. However, practically it is not easy to increase the number of areas to be surveyed due to the limitation of man power, and it is considered that the general trend of the seasonal or yearly change in mosquito density can be given even by such data.

It was clearly demonstrated in the present paper that the number of human cases depends on the density of *tritaeniorhynchus* during the epizootic in pigs in the Nagasaki district (Fig. 6), as reported for other areas in Japan (Buei et al., 1968; Ishida et al., 1969; Yamamoto, 1971). The size of human epidemic in Nagasaki was greatest in 1966, and became small remarkably after that year. The immune status in the human population would also influence the number of cases. The change in the immune status during the ten years of the present study is only very imperfectly known. However, the fact that the rate of pigs positive for HI antibody remained far below a 100% level in 1972 suggested that a very small number of infective mosquitoes were produced. Therefore, it seems certain that the lowering density of *tritaeniorhynchus* is one of the most important factors to have induced the reduction of the number of human cases in recent years.

Ishida et al. (1969) reported that the epizootic in pigs starts after the density of vector mosquitoes exceeds a certain threshold value in the Tohoku district, north Japan, and Wada (1975) suggested the presence of the threshold vector density by theoretical consideration. This seems true for many years in the Nagasaki district, too (Fig. 3). However, the density of *tritaeniorhynchus* might be lower in 1972 and 1973 than the supposed threshold level for JE epizootic to start, nevertheless pig infections occurred. The reason may be that the vector density was high enough for the epizootic in some areas, other than Kaizu and Mogi, and the infected mosquitoes dispersed to the present study areas and the pig epizootic occurred in a small scale, because *tritaeniorhynchus* has a strong ability to fly (Wada et al., 1969). It may be reasonable to suppose that the JE epizootic will occur, if the vector density exceeds the threshold value at time of the introduction of the virus into the pig - mosquito cycle.

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日本における日本脳炎ウイルスの生態学 II. 伝搬蚊の個体群と日本脳炎の流行

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1964年から1973年の間に長崎地方で得られた資料を用いて, 伝搬蚊コガタアカイエカの個体群と日本脳炎の流行との関係を吟味した. その結果次の諸点が明らかとなった. (1)各年のコガタアカイエカの越冬雌成虫及び新生雄成虫の出現開始時期と, 日本脳炎の流行が始まる時期との間には関係は認められなかった. (2)日本脳炎の流行の開始は, 一般にはコガタアカイエカ個体群の密度の上昇と一致していた. (3)日本脳炎患者の発生数が多い年には, コガタアカイエカの1年を通じての累積密度は一般に高かった. しかし, 伝搬蚊の累積密度がある特定の値より低い年には, 極めてわずかの患者しか発生しなかった. この累積密度の特定の値は, おそらく豚で日本脳炎が流行するための伝搬蚊の限界密度と関連したもののように思われる. (4)日本脳炎患者数は, 豚で流行が起っている時期の伝搬蚊の密度と明らかに比例関係にあることがわかった. (5)日本脳炎ウイルスによるコガタアカイエカの感染率は年によって違いがあるが, 日本脳炎の流行の規模とは無関係であった. (6)越冬からさめたばかりのコガタアカイエカ雌成虫は何れも未経産であったので, 越冬コガタアカイエカの体内で日本脳炎ウイルスが越冬する可能性はまず無いと考えられた. (7)近年におけるコガタアカイエカの密度の低下は, 患者の発生数の減少をもたらした最も重要な要因の1つと考えられた.

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