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Ecological Studies on Japanese Encephalitis Virus: Results of Investigations in Nagasaki Area, Japan, in 1969, 1970 and 1971

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Abstract

Serial surveys on the ecology of Japanese encephalitis virus in Nagasaki area were made during from 1969 to 1971. In total, 11,229 hibernated female mosquitoes of *Culex tritaeniorhynchus* were caught in early spring every year for virus isolation, however it was unsuccessful. The significant increase of hemagglutination inhibition antibody possessing rate in the sera of slaughtered pigs in early spring was not found in these three years. Virus isolations from the vector mosquitoes in epidemic season were made from 1st to 26th of August in 1969, from 19th of July to 16th of August in 1970 and from 13th to 27th of July in 1971, respectively. Although the isolation efficiencies were not remarkably different at the highest level in a certain limited period in epidemic season during the years 1964 to 1971, the periods for JE virus isolation from the vector mosquitoes became shorter in the years from 1968 to 1971 than from 1964 to 1967. It was considered as one of the reasons that the number of the vector mosquitoes was smaller during the epizootic from 1968 to 1971 than from 1964 to 1967. Subsequently, it was noted that the encephalitis cases became to decrease in number in recent years.

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Introduction

Attempts to isolate the virus from *Culex tritaeniorhynchus* mosquitoes caught in early spring and also in epidemic season in Nagasaki area were continued since 1964. In connection with the virus isolation from them, the development of hemagglutination inhibition antibody against Japanese encephalitis (JE) virus in the sera of slaughtered pigs during preepizootic and epizootic seasons and the occurrence of human incidence of JE were investigated at the same time. In this paper, the results of investigation on the ecology of JE virus in 1969, 1970 and 1971 will be described.

Materials and Methods

1. Places and methods for mosquito collection

As shown in Fig. 1, mosquitoes were collected at Mogi and Kaizu near Nagasaki city in all the years from 1969 to 1971, and also at Hongochi in the suburb of the city in 1969 and 1970.

Periods and times for the mosquito collection were presented in Table 1. In early spring, hibernated female mosquitoes of *Culex tritaeniorhynchus* (C.t.) were caught by using dry ice in the field and by an aspirator at pigsties as described in the previous paper by Omori et al. (1965).

2. Virus isolation and identification

Virus isolation from mosquitoes was made by intracerebral inoculation of supernatant of mosquito suspension into suckling mice as described in the previous paper by Hayashi et al. (1965) and viruses isolated were identified by the exami-

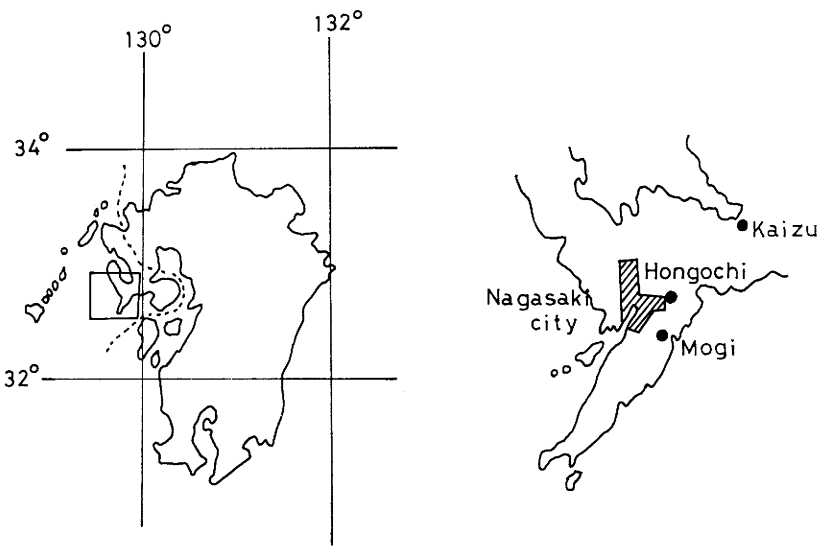

Fig. 1. Outline of Kyushu Island and Nagasaki Prefecture and Places for Mosquito Collection in Nagasaki Area.

nation of hemagglutination (HA) activity at the appropriate pH range and of hemagglutination inhibition (HI) or neutralization (NT) using the antiserum against JaGAr 01 strain of JE virus.

3. HI test of human and Swine sera

After the treatment of the sera with aceton, the examination for total HI and 2-mercaptoethanol (2-ME) sensitive antibodies was made as appeared in the previous paper by Hayashi et al. (1965, 1966).

Table 1. Period for mosquito collection in Nagasaki area

Year	Village	Period for collection (days)	Times of collection
1969	Kaizu	March, 19 – October, 27 (223)	63
	Mogi	March, 24 – October, 31 (222)	54
	Hongochi	June, 27 – September, 25 (91)	6
1970	Kaizu	February, 14 – October, 29 (258)	52
	Mogi	February, 14 – October, 22 (246)	41
	Hongochi	May, 14 – August, 27 (106)	4
1971	Kaizu	February, 22 – October, 26 (247)	34
	Mogi	February, 22 – October, 26 (247)	34

Results

1. Results in 1969

(1) Seasonal prevalence of C. t. mosquitoes and virus isolation from them

The number of C.t. mosquitoes reached the highest level in the late part of July and/or in the early part of August and it decreased remarkably in the late part of September as seen in Fig. 2.

As shown in Table 2, from the late part of March to the middle part of May, 7,712 hibernated female mosquitoes of C.t. were caught in the fields. An attempt to isolate the virus from them in 42 pools was unsuccessful. The first virus isolation from C. t. mosquitoes was made on 1st of August and the latest one on 26th of August. The period for virus isolation from mosquitoes of C. t. in epidemic season was 26 days. Table 3 was given

Table 2. JE virus isolation from *C. tritaeniorhynchus* in Nagasaki area, 1969.

Month		No. of mosquitoes	No. of pools examinde	No. of pools positive	Isolation efficiency
March	L	49	1	0	0
April	E	83	1	0	0
	M	6,453	34	0	0
	L	1,023	6	0	0
May	E	104	2	0	0
	M	0	0	0	0
	L	1,013	13	0	0
June	E	1,132	5	0	0
	M	2,030	11	0	0
	L	1,701	7	0	0
July	E	77	1	0	0
	M	289	2	0	0
	L	2,277	9	0	0
August	E	3,344	16	10	3.0
	M	1,937	9	7	3.6
	L	1,603	10	3	1.8
September	E	505	4	0	0
	M	142	3	0	0
	L	0	0	0	0
Total		23,951	134	20	0.8

Remarks: The signs of E, M, and L mean the early, middle, and late part of a month.

the results that 134 pools consisting of 23,951 mosquitoes of C. t. including hibernated females caught in early spring were tested for the virus isolation and 20 strains isolated were identified as JE virus.

(2) Swine infection and human cases

The 2ME sensitive antibody in the sera of slaughtered pigs was detected at the

Table 3. JE virus isolation from mosquitoes by species in Nagasaki area, 1969.

Species	Collection period	No. of mosquitoes	No. of pools	No. of positive pools
An. lindesayi japonicus	Apr. 22	4	1	0
An. sinensis	Mar. 19 − Sept. 25	7,878	50	0
An. sineroides	Apr. 22	22	1	0
Ar. subalbatus	May 2 − Sept. 5	2,558	18	0
Ae. japonicus	Apr. 22	6	1	0
Ae. vexans nipponii	Apr. 11 − Sept. 5	5,124	35	0
C. bitaeniorhynchus	Aug. 1	40	1	0
C. pipiens pallens	May 7 − Sept. 2	323	5	0
C. pseudovishnui	Apr. 13 − Aug. 19	183	8	0
C. triteaniorhynchus	Mar. 24 − Sept. 25	23,951	134	20
C. whitmorei	Apr. 22	45	1	0
Total		40,134	255	20

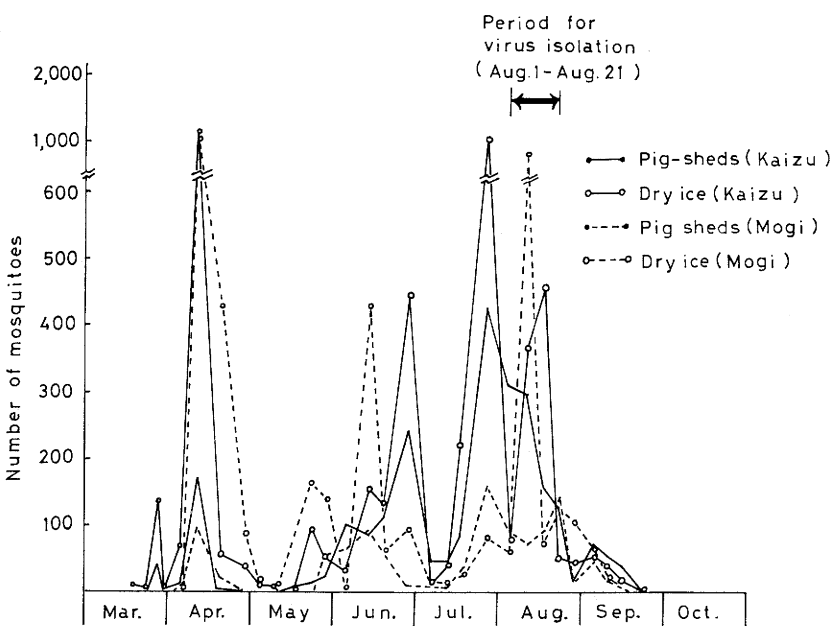

Fig. 2. Seasonal Prevalence of *Culex tritaeniorhynchus* Mosquitoes Collected in the Fields or at the Pig-sheds in Nagasaki Area and the Period for JE Virus Isolation in 1969.

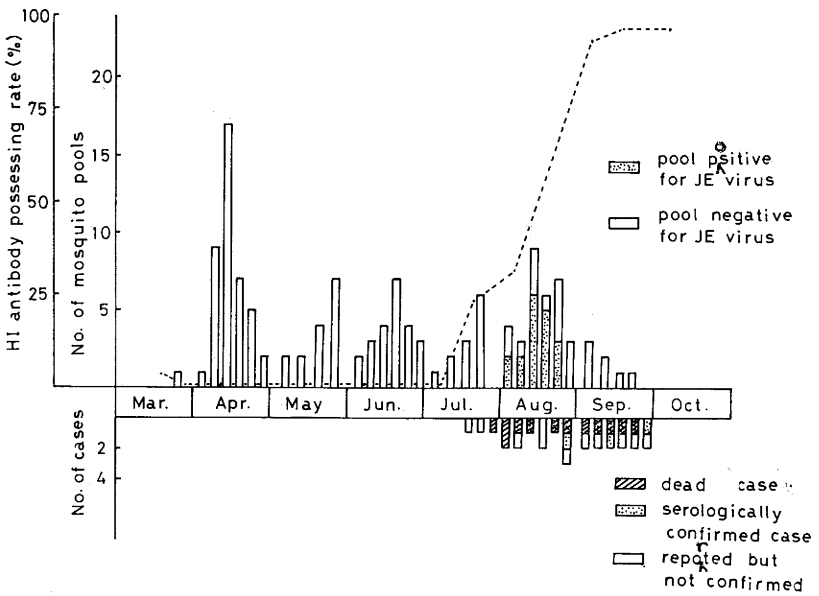

Fig. 3. JE virus isolation from mosquitoes of *C. tritaeniorhynchus* in relation to antibody rising in swine sera and to outbreak of encephalitis case in Nagasaki area, 1969.

level of 24.1 percent on 24th July and the possessing rate of total HI antibody reached to the level over 90 percent in the early part of September.

Twenty six cases of encephalitis were reported from 22nd of July to 26th of September and 11 out of them were confirmed serologically as genuine cases (Fig. 3).

II. Results in 1970

(1) *Seasonal prevalence of C.t. mosquitoes and virus isolation from them*

The pattern of seasonal prevalence of C.t. mosquitoes given in Fig. 4 was similar to that in 1969.

As seen in Table 4, 7 pools consisting of 1,225 mosquitoes of hibernated C.t., of which number was smaller than in 1969, were examined for the virus isola-

Table 4. JE virus isolation from *C. tritaeniorhynchus* in Nagasaki area, 1970.

Month		No. of mosquitoes	No. of pools		Isolation efficiency
			examined	positive	
April	M	1,038	4	0	0
	L	44	1	0	0
May	E	31	1	0	0
	M	112	1	0	0
	L	195	3	0	0
June	E	270	2	0	0
	M	803	5	0	0
	L	792	4	0	0
July	E	895	3	0	0
	M	629	3	1	1.6
	L	1,674	8	8	4.8
August	E	905	4	3	3.3
	M	477	3	2	4.2
	L	412	4	0	0
Total		8,277	46	14	1.7

Remarks: See Table 2.

tion and no virus was detected from them. In epidemic season, the virus isolation from vector mosquitoes was made from 19th of July to 16th of August. In total, 14 strains of JE virus were isolated from 46 pools consisting of 8,277 mosquitoes of C.t. including hibernated females as shown in Table 5. On 7th of August, 2 strains of non-JE viruses were isolated from a pool consisting of 60 mosquitoes of *Aedes vexans nipponii* and a pool consisting of 109 mosquitoes of *Armigeres subalbatus* respectively. These viruses were not yet identified, but they have not the activity of HA at the pH range from 6.0 to 7.2, and further examination are being studied.

Table 5. JE virus isolation from mosquitoes of different species in Nagasaki area, 1970.

Species	Collection period	No. of mosquitoes	No. of pools	No. of positive pools
Ar. subalbatus	May 7 – Aug. 7	130	4	0(1)
Ae. vexans nipponii	Apr. 15 – Aug. 7	963	10	0(1)
C. pipiens pallens	May 7 – Jun. 4	75	2	0
C. tritaeniorhynchus	Apr. 15 – Aug. 27	8,277	46	14
Total		8,435	62	14(2)

( ) : non-JE arbovirus

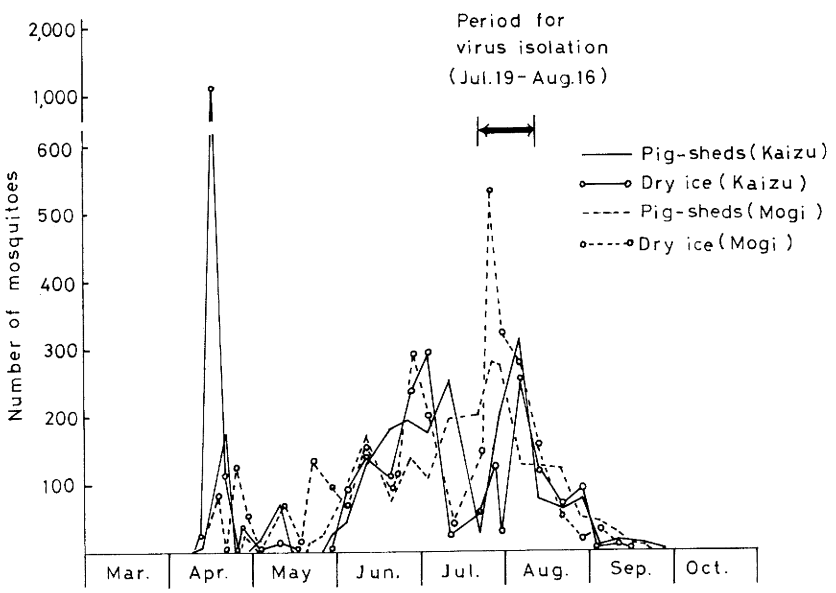

Fig. 4. Seasonal prevalence of *Culex tritaeniorhynchus* mosquitoes collected in the fields or at the pig-sheds in Nagasaki area and the period for JE virus isolation in 1970.

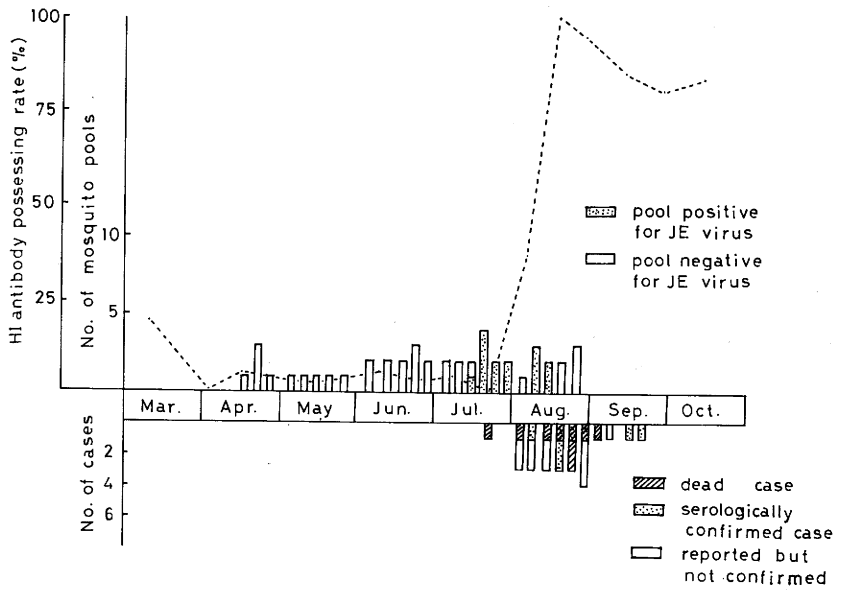

Fig. 5. JE virus isolation from mosquitoes of *C. tritaeniorhynchus* in relation to antibody rising in swine sera and to outbreak of encephalitis case in Nagasaki area, 1970.

(2) Swine infection and human cases

The HI antibody possessing rate in the sera of slaughtered pigs began to rise in the early part of August and reached to a level of about 100 percent on 11th of August. More abruptly rising pattern was shown than in 1969 (Fig. 5).

Twenty four cases of encephalitis were reported from 23rd July to 18th of September and 8 out of them were serologically confirmed.

III. Results in 1971

(1) Seasonal prevalence of C. t. mosquitoes and virus isolation from them

An attempt to isolate the virus from 22 pools consisting of 2,744 hibernated female mosquitoes of C.t. caught in early spring was unsuccessful. Table 6

Table 6. JE virus isolation from *C. tritaeniorhynchus* in Nagasaki area, 1971.

Month		No. of mosquitoes	No. of pools examined	No. of pools positive	Isolation efficiency
March	L	217	4	0	0
April	E	1,516	7	0	0
	M	731	5	0	0
	L	75	2	0	0
May	E	0	0	0	0
	M	205	4	0	0
	L	41	1	0	0
June	E	391	5	0	0
	M	299	3	0	0
	L	890	6	0	0
July	E	508	4	0	0
	M	1,373	8	6	4.4
	L	504	4	2	4.0
August	E	0	0	0	0
	M	269	3	0	0
	L	17	1	0	0
Total		7,036	57	8	1.0

Remarks: See Table 2.

was given the result that JE viruses were isolated from vector mosquitoes from 13th of July to 27th of July. Despite of the pattern of seasonal prevalence of C.t. mosquitoes was similar to those in the previous two years as shown in Fig. 6, the period for JE virus isolation was shorter than in 1969 and 1970. As seen in Table 7, only 8 strains of JE virus were isolated from 57 pools consisting of 7,036 mosquitoes of C.t. including hibernated ones caught in early spring.

Table 7. JE virus isolation from mosquitoes of different species in Nagasaki area, 1971.

Species	Collection period	No. of mosquitoes	No. of pools	No. of positive pools
An. sinensis	Feb. 22	1,138	5	0
Ar. subalbatus	Apr. 27 – Aug. 18	170	5	0
Ae. vexans nipponii	Apr. 13 – Aug. 24	484	12	0
C. bitaeniorhynchus	May 25 – Jun. 1	34	2	0
C. pipiens pallens	May 18 – Jun. 1	159	4	0
C. pseudovishnui	Aug. 12	3	1	0
C. tritaeniorhynchus	Mar. 29 – Aug. 24	7,036	57	8
Total		9,024	86	8

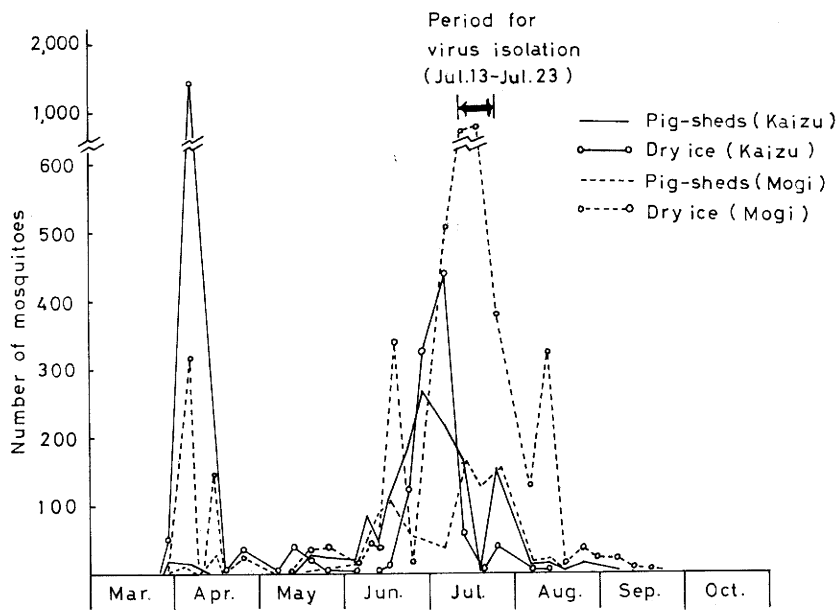

Fig. 6. Seasonal prevalence of *Culex tritaeniorhynchus* mosquitoes collected in the fields or at the pig-sheds in Nagasaki area and the period for JE virus isolation in 1971.

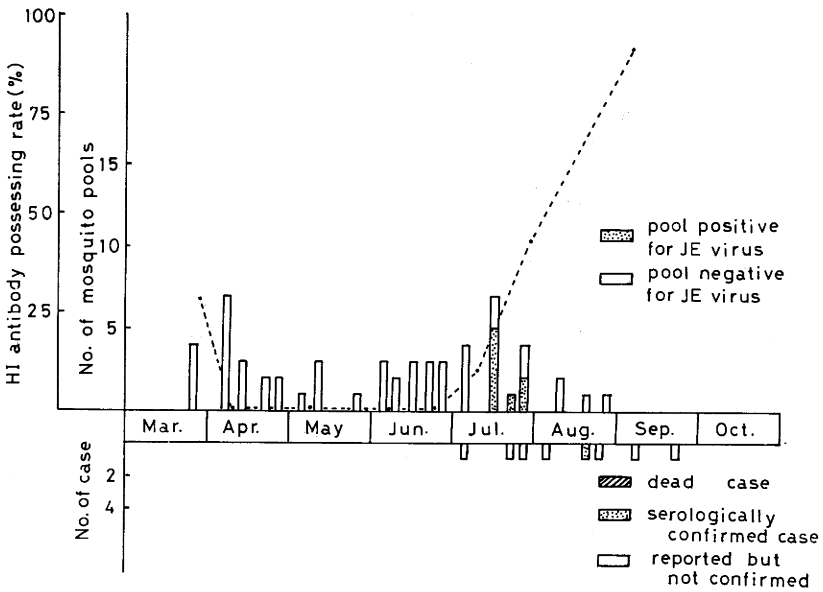

Fig. 7. JE virus isolation from mosquitoes of *C. tritaeniorhynchus* in relation to antibody rising in swine sera and to outbreak of encephalitis case in Nagasaki area, 1971.

(2) Swine infection and human cases.

The HI antibody possessing rate in the sera of slaughtered pigs rised gradually from 6th of July to 7th of September when it reached a level of 92.0 percent. The rising pattern of HI antibody possessing rate was similar to that in 1969.

In parallel with the shortest period for the virus isolation from the vector mosquitoes in previous several years, only 8 incidences of encephalitis were reported in this year and one out of them was serologically confirmed as a genuine case (Fig. 7).

Discussion

An attempt to isolate the virus from pools consisting of 86,128 hibernated female mosquitoes of C.t. caught in early spring from 1965 to 1971 including 73 pools of 11,681 mosquitoes collected in recent three years was unsuccessful by the method of intracerebral inoculation into suckling mice. At the same time, the careful examination for the 2ME sensitive antibody in the sera of slaughtered pigs in early spring was performed,

Table 8. Periods of JE virus isolation from mosquitoes of *Culex tritaeniorhynchus* caught in Nagasaki area.

Year	First and last day of virus isolation	Period of virus isolation (days)
1964	June, 8 – August, 7	61
1965	May, 30 – September, 6	100
1666	June, 24 – August, 27	65
1967	June, 23 – July, 27	35
1968	July, 22 – August, 7	17
1969	August, 1 – August, 26	26
1970	July, 19 – August, 6	28
1971	July, 13 – July, 27	14

but it was not detected in these three years.

Table 8 shows the summarized results of virus isolation from the vector mosquitoes in epidemic season in Nagasaki area from 1964 to 1971. Despite of the fact that there was no remarkable difference in the values of JE virus isolation efficiency in a certain limitted period in every epidemic season, the period for the virus isolation from 1968 to 1971 became shorter than that from 1964 to 1967. It was suggested that JE virus dissemination in nature from 1968 to 1971 became to be smaller than from 1964 to 1967. It was also considered as one of the reasons that the number of the vector mosquitoes was smaller during the epizootic season in recent four years than in 1964 to 1967. Consequently, this must have produced the result that the cases of encephalitis remarkably decreased in number from 1968 to 1971.

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日本脳炎ウイルスの生態学の研究：1969年，1970年及び1971年の調査成績

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摘　　要

1969年，1970年及び1971年の3年間に越年コガタアカイエカ 11,229個体，65 プールについて哺乳マウス脳内接種法でウイルスの分離を試みたが，成績は陰性であった．流行期における蚊からのウイルスの分離は 1969年は，8月1日から8月26日まで，1970年は，7月19日から8月6日まで，1971年は7月13日から7月27日までの期間であった．各年の捕集蚊からの日脳ウイルスの分離効率は最も高いときは 1969年 3.6，1970年 4.8，1971年 4.4 であって，1968年以前の流行盛期のそれと大差がない．以上の事実は，最近3年間の野外でのコガタアカイエカのウイルス汚染が流行期の或る一時期には 1968年以前と同じくらいに行われていることを示している．これに反して，蚊からのウイルスの分離期間が異常に短かくなっていることは，コガタアカイエカの発生，消長が最近3年間では著しく減少していることに，その一つの要因を求めることが出来る．これと平行して．患者数も減少し，1969年 19名，1970年 17名，1971年 3名の届出患者があったにすぎない．