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Ecological Studies on Japanese Encephalitis Virus
Results of investigations in the Nagasaki area, Japan, in 1968

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Abstract

A serial survey on the ecology of Japanese encephalitis (JE) virus was made in 1968. Virus isolation was negative from 848 hibernated females of *Culex tritaeniorhynchus* collected in March to early May. In mid May, when newly emerged females appeared, and thereafter, attempts to isolate the virus were continued, but it was July 23 that the first isolation was made. A strain of JE virus was isolated each from *C. pseudovishnui* in early August and from *Aedes vexans nipponii* in late July. Eight other species of mosquitoes were negative for JE virus throughout the year. The pigs susceptible to JE virus were exposed in nature to mosquitoes including hibernated females of *C. tritaeniorhynchus* in spring without detecting the rise in hemagglutination inhibition (HI) antibody in their sera. Continuing the exposure to mosquitoes, the HI antibody was detected only after early August. The number of human cases was smaller than in any of the previous three years. One of the reasons is considered to be that the number of vector mosquitoes was smaller at the time of the epizootic in pigs.

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Introduction

In spite of the experimental evidence that Japanese encephalitis (JE) virus can overwinter in infected *Culex tritaeniorhynchus* females (Mifune, 1965), careful and extensive attempts to isolate the virus from overwintered mosquitoes in the field have been unsuccessful since 1965. However, it is an interesting fact that a few pigs were found positive for hemagglutination inhibition(HI) antibody sensitive to 2-mercaptoethanol (2-ME) against the virus from mid April to early May in 1967 (Hayashi et al., 1968). This seems to mean that the pigs were infected in spring when the new generation of *C. tritaeniorhynchus* had not yet appeared. Thus, it was thought to be worthwhile to repeat the attempts to isolate the virus from collected mosquitoes and to detect the HI antibody in susceptible pigs exposed to mosquitoes, in spring around Nagasaki City, including the places where the pigs with HI antibody sensitive to 2-ME had been detected in 1967.

Besides the above, the attempts to isolate the virus from mosquitoes and to detect the HI antibody in the susceptible pigs were continued till late autumn, and the survey for the rising of HI antibody in pig sera was made from early spring to late autumn, to analyse the epidemiological features of JE in the Nagasaki area.

Methods

Mosquitoes were collected from early spring to late autumn by dry ice traps and at pig- and cow-sheds at the five villages of Kobasaki, Koebaru, Nishiyama, Mogi, and Kaizu at a regular interval of one week, and at the three villages of Nunomaki, Tomachi, and Hongochi at an irregular interval. The methods for the collection of mosquitoes were the same as described by Wada et al. (1967). All the villages are located around Nagasaki City. Koebaru and Nishiyama, where the pigs with 2-ME sensitive HI antibody were found in spring in 1967, are located between small hills, and paddy-fields are developed there only to a very small extent. The other villages are as described by Wada et al. (1967).

Collected mosquitoes were identified and pooled for the isolation of virus. The procedure for the virus isolation from mosquitoes and the identification of isolated virus are the same as given by Hayashi et al. (1965).

The HI antibody against JE virus was examined from early spring to late autumn in the sera of pigs slaughtered in the Nagasaki area and also those being susceptible to the virus placed in the three villages of Nishiyama, Mogi, and Kobasaki. The susceptible pigs were sent from Obihiro City in Hokkaido, which is free from the epidemic of JE throughout the year, and placed on February 29, 1968 in the pig-sheds.

In this paper, the HI antibody sensitive to 2-ME treatment is called 2-ME sensi-

antibody, while the HI antibody in the untreated sera is referred to as total HI antibody. Those antibodies were examined by the method described by Hayashi et al. (1965, 1966) and Konno et al. (1967).

Results

Seasonal prevalence of C. tritaeniorhynchus females

Seasonal prevalence of *C. tritaeniorhynchus* females collected by dry ice traps and at animal-sheds are shown in Figs. 1 and 2, respectively. The overwintered females were firstly collected by a dry ice trap at Kaizu on March 21.

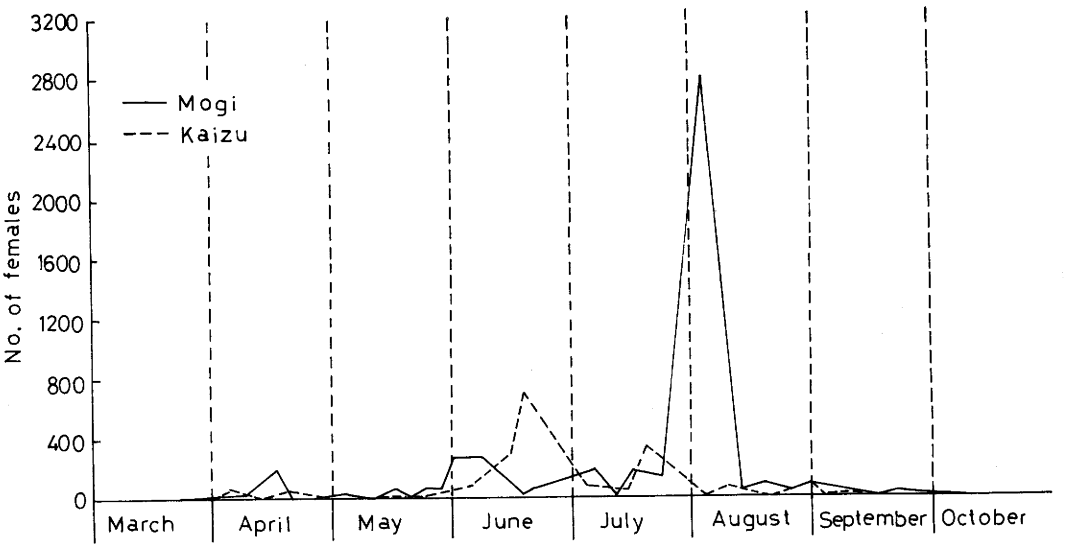

Fig. 1. Seasonal prevalence of *C. tritaeniorhynchus* females collected by dry ice traps in the Nagasaki area, 1968.

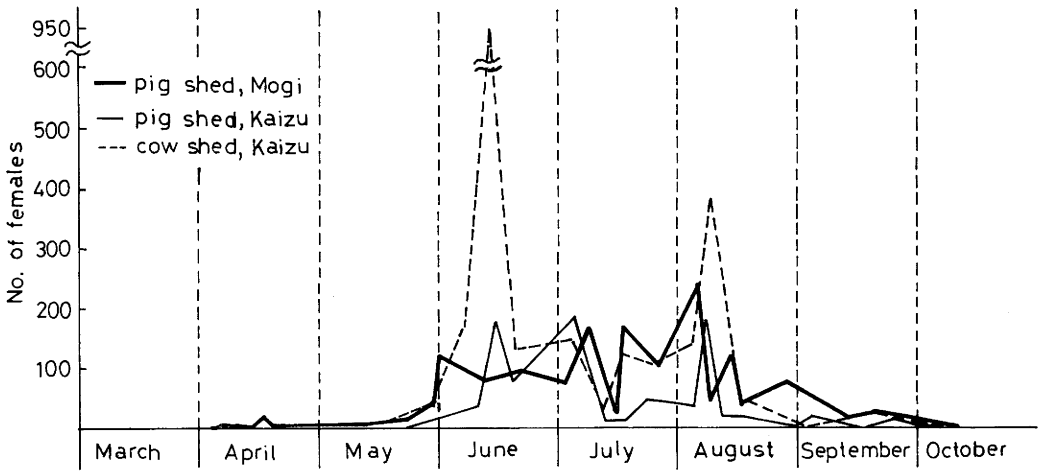

Fig. 2. Seasonal prevalence of *C. tritaeniorhynchus* females collected at animal-sheds in the Nagasaki area, 1968.

The first males, which indicate the emergence of a new generation, were encountered in a swarm at Mogi on May 14. As for the seasonal prevalence, although the patterns are not necessarily the same by village and method as shown in Fig. 1 and 2, the general trend can be said as follows : the number of overwintered females was very small ; newly-emerged females were also small in number, with the two peaks in mid June and early August. When compared with the breeding numbers of this mospuito in the previous three years, the number in 1968 was fairly smaller throughout the year.

Virus isolation from mosquitoes

Table 1. Virus isolation from mosquitoes by species collected by various methods in the Nagasaki area, from early spring to late autumn, 1668.

Mosquito species	No. of mosqs.	No. of pools	
		examined	positive
An. lindesayi japonicus	5	1	0
An. sinensis	5,964	58	0
An. sineroides	94	2	0
Ar. subalbatus	1,668	13	0
Ae. albopictus	39	3	0
Ae. vexans nipponii	4,038	29	1
C. bitaeniorhynchus	17	1	0
C. pipiens pallens	628	12	0
C. pseudovishnui	152	5	1
C. tritaeniorhynchus	7,272	74	6
C. whitmorei	52	2	0

Table 2. Seasonal appearance of the positive pools for the virus in *Culex tritaeniorhynchus*, *C. pseudovishnui*, and *Aedes vaxans nipponii* shown in Table 1.

Month		*C. tritaeniorhynchus*			*C. pseudovishnui*			*Ae. vexans nipponii*		
		No. of mosqs.	No. of pools		No. of mosqs.	No. of pools		No. of mosqs.	No. of pools	
			examined	positive		examined	positive		examined	positive
Mar.	L	56	1	0	–	–	–	–	–	–
	E	308	5	0	–	–	–	160	1	0
Apr.	M	236	3	0	19	1	0	616	3	0
	L	62	1	0	–	–	–	135	1	0
	E	186	4	0	–	–	–	40	1	0
May	M	153	2	0	–	–	–	144	1	0
	L	843	8	0	26	1	0	126	1	0
	E	648	8	0	–	–	–	241	3	0
Jun.	M	189	2	0	–	–	–	–	–	–
	L	282	2	0	–	–	–	28	1	0
	E	476	7	0	–	–	–	229	2	0
Jul.	M	462	3	0	–	–	–	70	1	0
	L	772	6	1	56	1	0	535	3	1
	E	1,619	10	5	51	2	1	554	2	0
Aug.	M	163	4	0	–	–	–	314	3	0
	L	696	6	0	–	–	–	63	3	0
	E	–	–	–	–	–	–	–	–	–
Sep.	M	91	1	0	–	–	–	135	1	0
	L	30	1	0	–	–	–	287	1	0
Oct.	E	–	–	–	–	–	–	361	1	0

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

Table 1 gives the results of the virus isolation from female mosquitoes collected by various methods in the Nagasaki area from early spring to late autumn. It is shown in Table 1 that the virus was isolated from three species of mosquitoes, *C. tritaeniorhynchus*, *C. pseudovishnui*, and *Ae. vexans nipponii*. The number of positive pools for virus was six out of 74 pools consisting of 7,272 *C. tritaeniorhynchus* females, one out of five pools of 152 *C. pseudovishnui*, and one out of 29 pools of 4,038 *Ae. vexans nipponii*. The isolated virus was all identified as JE virus by neutralization and HI tests by using standard antisera. The other eight species of mosquitoes were negative for JE virus. Thus, it was again confirmed that *C. tritaeniorhynchus* is the most important mosquito in the epidemiology of JE, at least in the epidemic season.

Table 2 gives the seasonal appearance of the positive pools for the virus in *C. tritaeniorhynchus*, *C. pseudovishnui*, and *Ae. vexans nipponii*: The 848 females of *C. tritaeniorhynchus* collected from late March to early May, which were all hibernated ones, were negative for the virus; the virus was isolated from *C.*

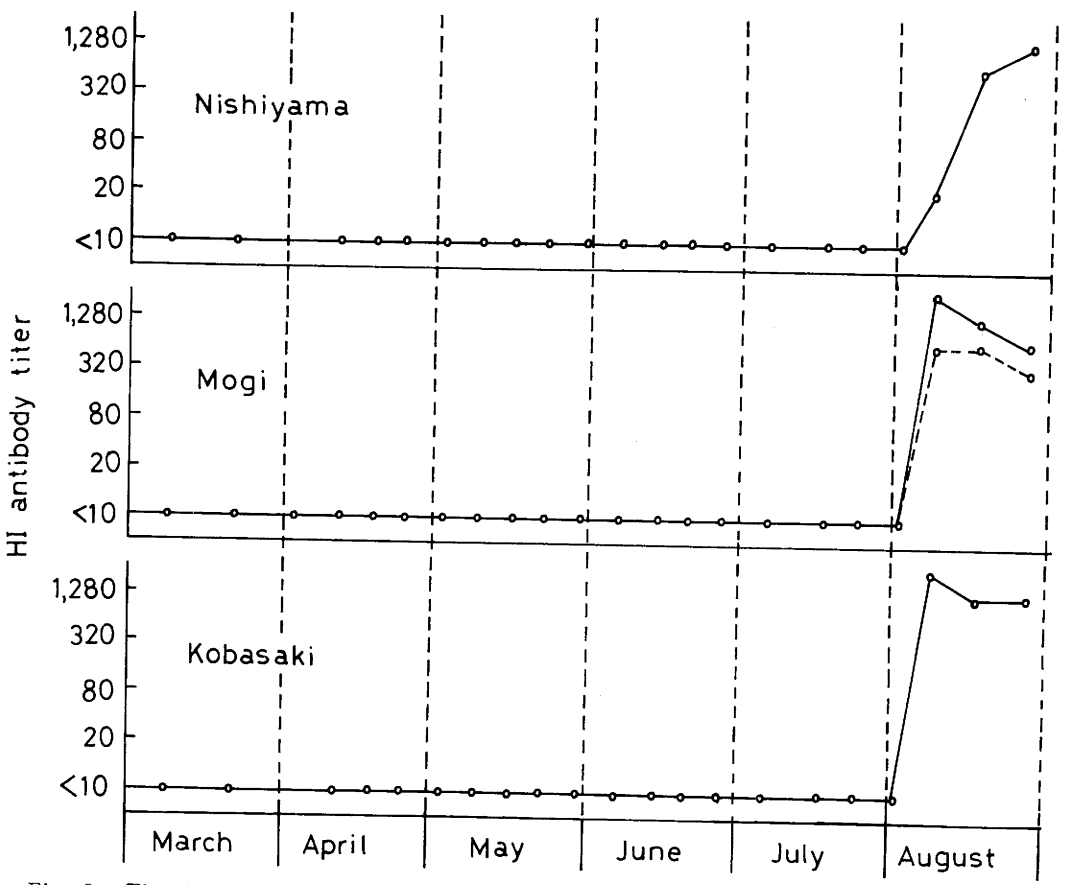

Fig. 3. The rising of HI antibody titer in the sera of the susceptible pigs kept at pig-sheds in the three villages of Nishiyama, Mogi, and Kobasaki in the Nagasaki area, 1968. Solid and dotted lines mean the titers of HI antibody in the sera untreated and treated with 2-ME, respectively.

Table 3. HI antibody rising in sera of pigs slaughtered in the Nagasaki area, 1968.

Month	No. and % of pigs			
	tested	positive	%	2-ME sensitive
Mar. L	175	9	5.1	0
E	106	4	3.8	0
Apr. M	127	9	7.1	0
L	137	9	6.5	0
E	138	4	2.9	0
May M	98	3	3.1	0
L	113	2	1.8	0
E	46	3	6.5	0
Jun. M	105	3	2.9	0
L	138	2	1.4	0
E	72	1	1.4	0
Jul. M	72	3	4.2	2/3
L	76	16	22.2	16/16
E	53	11	20.8	10/11
Aug. M	55	53	96.4	21/51
L	54	45	83.3	14/45
E	28	28	100.0	2/28
Sep. M	—	—	—	—
L	44	42	95.5	0/42

The sings of E, M, and L mean the early, middle, and late parts of a month.

tritaeniorhynchus from July 23 to August 7, and from *C. pseudovishnui* and *Ae. vexans nipponii* on August 6 and July 22 respectively.

HI antibody in the susceptible pigs

The three pigs susceptible to JE virus were kept at pig-sheds and examined for HI antibody. Those pigs, which were sent from a JE-free area of Hokkaido, were placed on February 29, 1968 at pig-sheds in the three villages of Nishiyama, Mogi, and Kobasaki; Nishiyama is the village where 2-ME sensitive antibody was detected in pig sera in spring of 1967. The pigs were exposed to mosquitoes in nature and were bled every week for HI antibody. The results obtained were given in Fig. 3. However, contrary to the initial expectation of the demonstration of the pig infection in spring, the HI antibody could not be detected until early August.

Pig and human infections

The rising of HI antibody in the sera of pigs slaughtered in the Nagasaki area is given in Table 3. The total HI antibody was detected in the whole period from late March to late September, but the antibody found till early July was thought to be due to the infection in the previous year, as the 2-ME sensitive antibody was not demonstrated. After mid July, owing to the new infections demonstrated by the appearance of 2-ME sensitive antibody, the possessing rate of total HI antibody increased gradually and reached a level near 100 % in mid August.

Fig. 4 illustrates the seasonal appearances of the HI antibody in slaughtered pigs, of the human encephalitis cases, and of the virus isolated from the pools of *C. tritaeniorhynchus* females, in the Nagasaki area.

The possessing rste of total HI antibody in slaughtered pigs increased gradually from July 20 up to August 20 when the rate reached a level near 100 %. The 2-ME sensitive antibody in the pigs began to appear also on July 20, the possessing rate increased until August 20 and thereafter decreased toward late September.

HI antibody in the pig serum appears from one week after the infection with JE virus (Mifune, 1965), and the antibody is sensitive to 2-ME for 2 to 4 weeks

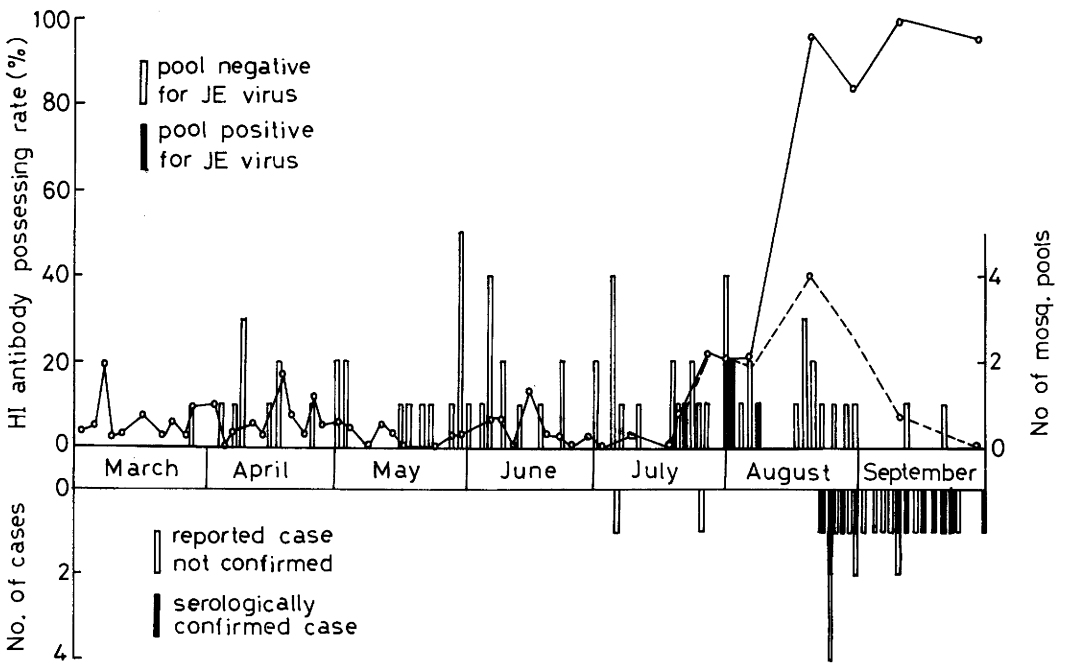

Fig. 4. The possessing rate of HI antibody in slaughtered pigs and the incidence of human encephalitis cases, together with thé virus isolation from the pools of *C. tritaeniorhynchus* females, in the Nagasaki area, 1968. Solid and dotted lines mean total HI antibody and 2-ME sensitive antibody respectively.

(Otsuka et al., 1967). In the present data, 2-ME sensitive antibody was firstly detected on July 20. This means that the pig infection in nature was started at least one week prior to July 20, namely on July 13 or slightly before. On the other hand, the possessing rate of 2-ME sensitive antibody in slaughtered pigs was 7.1 % on September 10 and 0 % on September 28, though the possessing rates of total HI antibody were 100% or nearly so. From this, it is considered that 2-ME sensitive antibody detected on September 10 may have started to appear, at earliest, on August 20, and the time of infection in nature must be one week before, that is about August 13. In this way, the period of the pig infection was estimated roughly as one month from July 13 to August 13. The mosquito infection from July 23 to August 7 ocurred within the period of the pig infection. This seems to suggest that the mosquitoes found positive for JE virus were infected from pigs in the viremic state.

The number of reported human encephalitis cases was 26, including 12 serologically confirmed cases having appeared from August 24 to September 30. The number of cases was smaller than in any of the previous three years. One of the reasons is considered to be that the number of vector mosquitoes was smaller during the pig epizootic.

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日本脳炎ウイルスの生態学的研究．1968年の調査成績

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摘　　要

前年までに引き続き，1968年に日本脳炎ウイルスの生態学的調査を行なった．３月－５月上旬に採集したコガタアカイエカ越冬雌成虫848個体からはウイルスは分離されなかった．新生雌成虫が出現し始めた5月中旬及びそれ以後も分離を試みたが，始めて日本脳炎ウイルスが分離できたのは７月23日であった．シロハシイエカからは８月上旬に，キンイロヤブカからは７月下旬に，各々１株の日本脳炎ウイルスが分離された．感受性の豚を各々１頭ずつ２月下旬に３部落の豚舎に配って，自然に蚊から吸血されるままにして飼育し続けて，HI抗体が出現する時期を調べたが，抗体が検出されたのは８月上旬以降であった．発生患者数は，過去３年の何れにおけるよりも少なかった．その理由の１つは，豚で日本脳炎の流行が起っている時期のコガタアカイエカの数が少なかったことであると考えられる．