Studies on Mosquito Infection with Japanese Encephalitis Virus in 1964 in Nagasaki Prefecture

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Abstract

In order to learn mosquito infection with Japanese encephalitis virus in relation to human epidemic of Japanese encephalitis in Nagasaki area, the virus isolation was made from mosquitoes collected in livestock pens mainly in Aino, Togitsu and Kaizu in Nagasaki-ken from May 19 to October 30, 1964.

A total of 33,187 female mosquitoes of 6 species (*Culex tritaeniorhynchus*, *Culex pipiens*, *Culex vishnui*, *Culex bitaeniorhynchus*, *Anopheles sinensis*, *Armigeres subalbatus*) were tested for virus isolations in 201 pools.

As the result, 30 out of 33 isolates were identified as Japanese encephalitis virus, and 3 were non-Japanese encephalitis virus. All strains of the isolated Japanese encephalitis virus came exclusively from *Culex tritaeniorhynchus*. The first mosquito infected with Japanese encephalitis virus was seen on May 19, in Aino. This is the earliest case of infected mosquito with Japanese encephalitis virus reported in Japan (including Okinawa) and Formosa. The viruses were continually isolated between May 19 and July 6, and in this period the infection rate of the mosquito was espetially high in late June in Aino.

On the other hand, the peak of *Culex tritaeniorhynchus* population was observed in the beginning of August and time of the occurrence of overt Japanese encephalitis patients reported in Nagasaki-ken was mid-July to the end of August. Accordingly the time discrepancy between the epidemic among mosquitoes and human in 1964 in Nagasaki-ken was remarkably different from that of the other places, such as Kanto area reported by previous investigators.

Among 3 strains of non-Japanese encephalitis virus, 2 were obtained from *Culex tritaeniorhynchus* collected on September 14, and one from a mixed pool of *Culex pipiens* and *Culex vishnui* collected during late July to mid-September.

Introduction

MITAMURA et al (1938; 1939) studied mosquito infection with Japanese encephalitis virus

for the first time in 1938 in Okayama area, Japan. Since then many ecological studies on Japanese encephalitis virus in relation to mosquitoes have been made in various part of Japan: Chiba, Saitama (BUESCHER et al., 1959), Gumma (MATSUYAMA et al., 1960; Oya et al., 1963; Oya et al., 1963—b), Fukuoka (YAMAMOTO et al., 1964), Osaka (ARAI et al., 1964), and Okinawa (HURLBUT et al., 1964), and in foreign lands: Formosa (WANG et al., 1962). As the result of these works, the time and size of human epidemics of Japanese encephalitis seem to be subjected to the influence of infected mosquitoes, espetially in the Kanto area. The fact that there have been no data published up to date as regards the infection of mosquitoes by the virus in the Nagasaki area prompted us to study the subject. The purpose of this paper is to present what we have found during the 1964 season.

Materials and methods

Area : Continual studies were carried out at Junte-buraku, Aino-machi, Minamitakagi-gun, Nagasaki-ken, which is located north-west of the Shimabara Peninsula, 36 kilo-

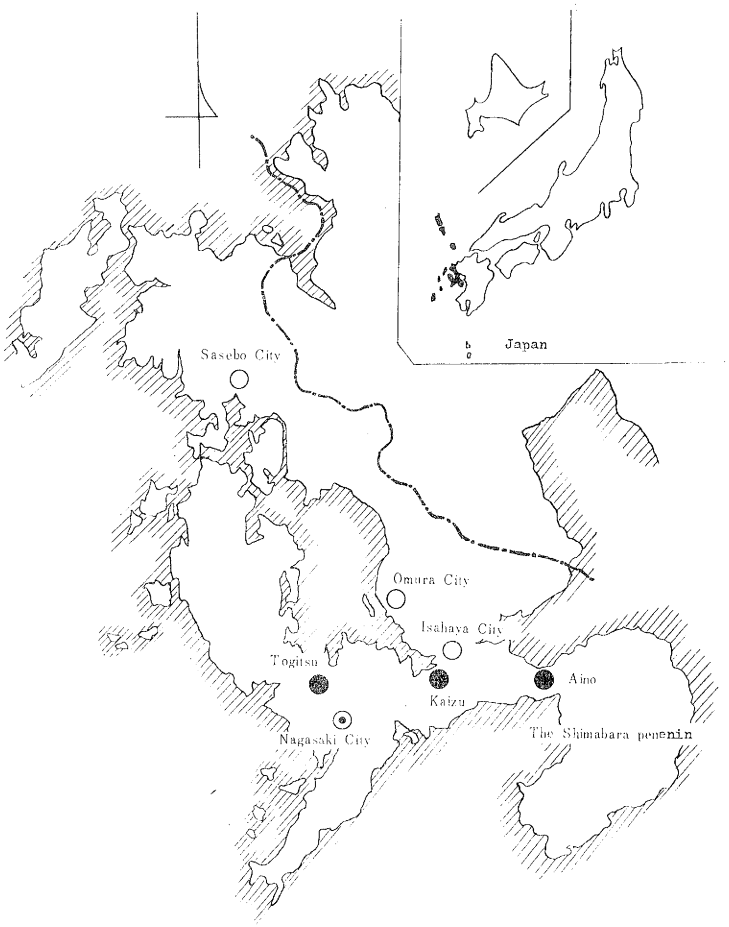

Fig. 1. Topography of Nagasaki prefecture.

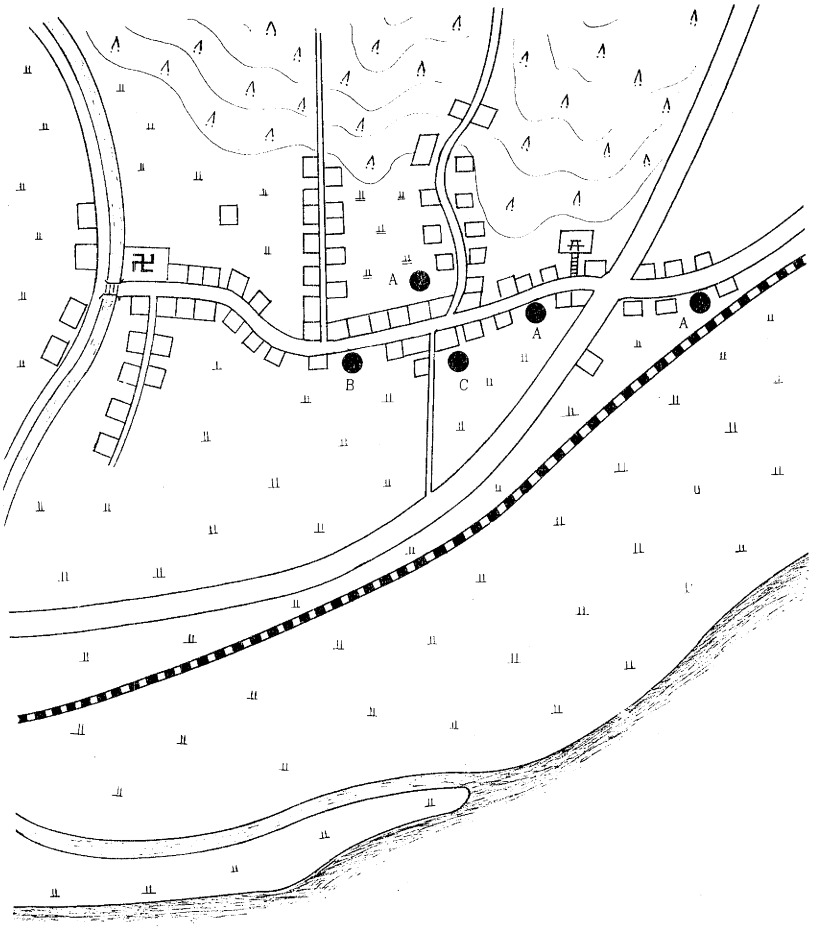

Fig. 2. The study site, Junte-buraku, Aino machi, Nagasaki prefecture. The solid circles show the places where the mosquitoes were collected.
A : cow barn, B : chicken coop, C : pig shed

meters from Nagasaki-shi. It is a farming village consisting of approximately 50 houses. There is a hill in the back and a vast rice field in front of the village. Almost all of the farmers in the village keep cows, pigs, chickens and goats. As show in Table 1, there are many susceptible vertebrates in the village, which have been born after the epizootics of Japanese encephalitis of the previous year. There was a human case of Japanese Encephalitis in the village in 1962.

We also made a temporary survey at Sasoko-buraku, Togitsu-machi and Nagasaki

Table 1. Population of domestic animals bved In Junte-buraku in Aino-machi in the 1964 season.
(in the end of August)

Species	Number of domestic animals		Total
	born before Octorber, 1963.	born aftev Octorber, 1963.	
Cow	52	12	64
pig	2	18	20
Chicken	1,087	1,004	2,091
Goat	2	1	3
Dog	6	7	13
Cat	17	26	43

Prefectural Institute of Experimental Agriculture and Forestry in Kaizu-machi. The former is located in the suburbs of Nagasaki-shi and the latter 25 kilo-meters east of Nagasaki-shi. These locations are shown in Fig. 1 and Fig. 2.

Collection of mosquitoes : The mosquitoes were collected from May 19, 1964 to Octorber 30, 1964.

During this period of time, mosquitoes were collected periodically from livestock pens in order to learn the seasonal prevalence of the vector mosquito in Aino. The mosquitoes were collected regularly in the cow barns, pig sheds and chicken coops by exhauster at a certain time (2 hours after sunset) and in a certain area. The collected mosquitoes were anesthetized by a mixed gas of Chloroform and dry ice, and classified under 20 timed magnifying glass or by the naked eye.

The classified mosquitoes were sealed in glass test tubes and kept in the dry ice box until the virus was to be isolated. Those operations were done immediately after the collection of the mosquitoes.

Isolation of the virus : The viruses were isolated according to the method used by the arbor virus study group of the National Institute of Health, Tokyo.

Pools of the adult female mosquitoes, which were classified according to species, date and place of collestion, were available for virus isolation. The interval between the collection of mosquitoes and the inoculation to mice ranged from 24 hours to 10 days. In general, a pool consisted of 200 mosquitoes. Some consisted of 100 and a few less than 100. The pools of mosquitoes were ground in a mortar and a solution was slowly added to make a suspension of triturated tissue. Volume of the diluent was varied according to the species: 2.0 cc. to each 100 *Culex tritaeniorhynchus*, 4.0 cc. to *Culex pipiens*, 2.0 cc. to *Culex vishnui*, 4.0 cc. to *Culex bitaeniohynchus*, 8.0 cc. to *Armigeres*, and 8.0 cc. to *Anopheles*. This diluent consists of buffer saline (pH 7.6) containing Penicillin 500 unit/cc and Streptomycin 500 microgram/cc, and in addition, 10% chick serum. The suspension was kept in the ice water bath for an hour and then centrifuged at the rate of 10,000 rpm for 30 minutes by cold centrifuging machine. A litter of suckling mice (gpc strain), 2 days to 5 days old, were inoculated intracerebrally with 0.02 cc. of each supernatant.

The inoculated mice were observed daily for 14 days for symptoms of central nervous system disease. The brain of those mice which became ill or died were removed and the brain tissue was reinoculated intracerebrally to other normal mice for isolation and identification of the virus. On the other hand, the brain tissue was also cultured in the thioglicolate media to determine whether there was any bacterial or fungal infection.

Identification of the virus : The viruses were identified by hemagglutination test and hemagglutination inhibition test. For hemagglutination test, sucrose aceton antigen was prepared from the diseased mouse brain at their third passage level. For hemagglutination inhibition test, the mouse immune sera against JaGAr #01 strain and Nakayama (NIH) starin were used.

Result

Species and number of collected mosquitoes : Total number of collected female mosquitoes amounted to 33,223 of 6 species : 27,230 in Aino, 3,633 in Kaizu, and 2,360 in Togitsu. As shown in Table 2 and 3, *Culex tritaeniorhynchus* was predominant in all of the livestock pens and was over 80% of all collected mosquitoes. *Anopheles sinensts* and

Table 2. Number anb species of mosquitoes collected at live stock pens in Nagasaki Prefecture in 1964.

Date collected		*Culex*				*Anopheles*	*Armigeres*	Total	Area
		tritaen.	*pipiens*	*vishnxi*	*bitaen.*	*sinensis*	*subalbatus*		
May	19	950	2	0	0	67	40	1,059	Aino
June	8	1,231	32	34	0	110	23	1,430	〃
	18	2,008	19	14	0	171	169	2,381	〃
	29	2,164	11	9	1	169	123	2,467	〃
July	6	3,261	16	23	3	618	57	3,978	〃
	16	1,792	39	11	1	220	38	2,101	〃
	27	1,672	5	7	3	357	89	2,133	〃
Aug.	4	3,040	13	32	2	138	15	3,240	〃
	11	3,528	9	34	0	81	23	3,675	〃
	18	654	17	12	0	104	43	830	〃
Sept.	1	1,417	10	17	2	146	32	1,624	〃
	14	1,237	42	24	0	57	4	1,364	〃
	30	132	87	1	0	108	212	540	〃
Oct.	14	135	83	0	0	32	71	321	〃
	30	0	7	0	0	0	81	88	〃
Total		23,211	392	218	12	2,377	1,020	27,230	
Aug.	7	3,448	1	184	0	0	0	3,633	Kaizu
	9	1,562	0	798	0	0	0	2,360	Togitsu
Grand total		28,221	393	1,200	12	2,377	1,020	33,223	

Table 3. Species and number of the mosquitoes collected by the source and recovery of Japanese encephalitis virus in Aino in 1964.

Mosquito source	*Culex tritaen.*		*Culex pipiens*		*Culex vishnui*		*Culex bitaen.*		*Anopheles sinensis*		*Armigeres subalbatus*		Total	
	No. of mosq.	No. of JEA	No. of mosq.	No. of JEV	No. of mosq.	No. of JEV	No. of mosq.	No. of JEV	No. of mosq.	No. of JEV	No. of mosq.	No. of JEV	No. of mosq.	No.
Cow barn	14,077	19	43	0	111	0	4	0	1,841	0	658	0	16,734	19
Pig shed	5,137	5	21	0	31	0	2	0	316	0	179	0	5,686	5
Chicken coop	3,047	2	326	0	76	0	6	0	153	0	143	0	3,751	2
Mixed	950	1	2	0	0	0	0	0	0	0	0	0	950	1
Total	23,211	27	392	0	218	0	12	0	2,377	0	1,020	0	27,230	27

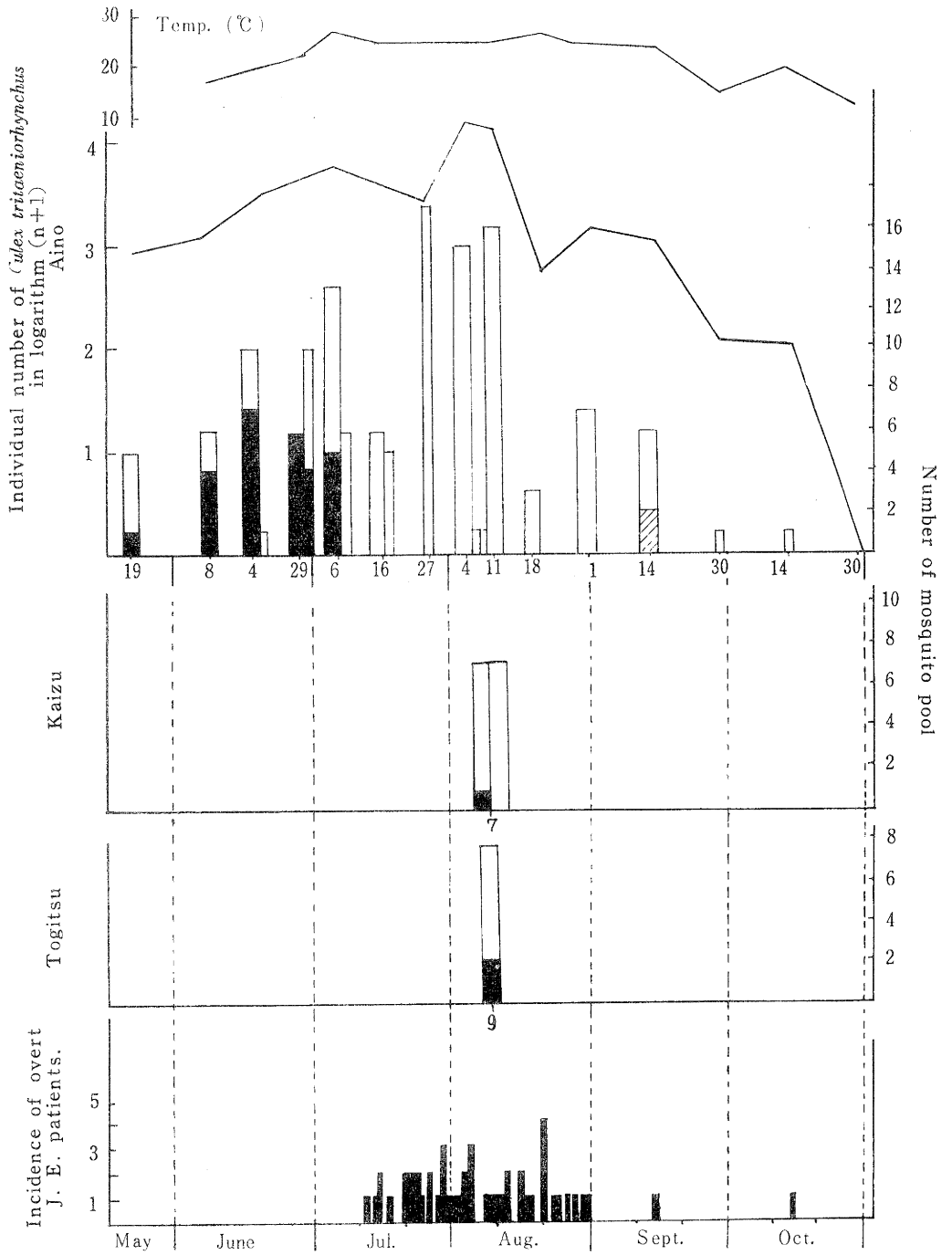

Fig. 3. Seasonal appearance and the incidence of the natural infection with Japanese encephalitis virus in *Culex tritaeniorhynchus* and the incidence of the overt Japanese encephalitis patients in Nagasaki prefecture in 1964.

Note : (1) ▭ : Pools containing approximately 100 mosquitoes.
(2) ▭ : Pools containing approximately 200 mosquitoes.
(3) The solid parts of rods show the number of the mosquito pools yielded the Japanese encephalitis viruses. The obliquely lined part of the rod shows the mosquito pools yielded non-Japanese encephalitis arbor virus.

Armigeres subalbatus were the next predominant group. *Culex vishnui* was particularly predominant in Togitsu. Most of *Culex pipiens* amounting to 83% were collected in the chicken coops suggesting the particular mosquito prefers poultry to mammals.

Seasonal prevalence of *Culex tritaeniorhynchus* : Seasonal prevalence of *Culex tritaeniorhynchus* during the survay is shown in Fig. 3. Although we do not know the population of the mosquito prior to the begining of our survey, we found that there were already considerable numbers of *Culex tritaeniorhynchus* present on May 19.

Since that time on the mosquitoes increased gradually in number toward July, and in early August the proliferation became so rapid as to form a peak. A transitional decrease seen in mid-July can be interpreted as being influenced by agricultural insecticides applied to the rice fields. After the peak in early August, *Culex tritaeniorhynchus* decreased rapidly towards September and there was none found at the end of Octorber. *Culex pipiens* did not shown remarkable changes in population.

Isolation of the Virus : The result of isolation of the virus from the 201 pools consisting of a total of 33,187 mosquitoes is shown in Table 4. Thirty-three strains of arbor viruses were isolated and 30 strains out of the 33 strains were identified as Japanese encephalitis virus and the remaining 3 strains were found to be quite different from the Japanese encephalitis virus in their serological and biological characteristics. Among those 3 strains, two srains were isolated from the pools of *Culex tritaniorhynchus* collected on September 14, and one strains was isolated from a mixed pool of *Culex pipiens* and *Culex vishnui* collected during the period begining late July to mid-September. The studies as to identify whether these 3 strains of virus are the same virus and belong to any particular group of arbor virus or not are now in progress. The

Table 4. Isolation of viruses from mosquitoes collected in Nagasaki Prefecture in 1964.

Species	Aino			Togitsu			Kaizu			Total		
	No. mosquitoes	No. pools	Virus isolation	No. mosquitoes	No. pools	Virus isolation	No. mosquitoes	No. pools	Virus isolation	No. mosquitoes	No. pools	Virus isolation
Culex tritaen.	23,211	135	29(2)	1.562	8	2	3.448	17	1	28.185	160	32(2)
Culex pipiens	392	11	1(1)*	0	0	0	1	1	0	393	17	1(1)*
Culex vishnui	218			798	4	0	184	1	0	1,200		
Culex bitaen.	12	1	0	0	0	0	0	0	0	12	1	0
Anopheles sinensis	2,377	15	0	0	0	0	0	0	0	2,377	15	0
Armigeres subalbatus	1,020	8	0	0	0	0	0	0	0	1.020	8	0
Total	27,230	170	30(3)	2,360	12	2	3,633	19	1	33,223	201	33(3)

Note : (1) Numbers in parenthesis denote the number of non-Japanese encephalitis virus among arbor viruses isolated from mosquitoes
(2)* ; Isolated from a mixed pool of *Culex pipiens* and *Culex vishnui* Comlex collected in July, Aug. and Sept.

Table 5. Isolation of Japanese encephalitis virus from *Culex tritaeniorhynchus* collected in Nagasaki area in 1964.

Date collected	Number of mosquitoes	Pools containing approximately 200 mosquitoes			Pools containing approximately 100 mosquioes			Isolation efficiency	Aera
		No. pools	Pools positive	Isolation rate	No. pools	Pools positive	Isolation rate		
May 19	950	5	1	20%				1.1	Aino
June 8	1,231	6	4	66%				3.3	〃
18	2,008	10	7	70%	1	0	0	3.5	〃
29	2,151	6	6	100%	10	4	40%	4.7	〃
July 6	3,261	13	5	38%	6	0	0	1.5	〃
16	1,792	6	0	0	5	0	0	0	〃
27	1,672				16	0	0	0	〃
Aug. 4	3,040	15	0	0	1	0	0	0	〃
11	3,528	17	0	0				0	〃
18	654	3	0	0				0	〃
Sept 1	1,417	7	0	0				0	〃
14	1,237	6	(2)	(33%)				(1.6)	〃
30	132				1	0	0	0	〃
Oct. 14	135				1	0	0	0	〃
30	0								〃
Total	23,211	94	25	27%	41	4	10%	1.3	〃
Aug. 7	3,448	17	1	6%				0.3	Kaizu
Aug. 9	1,562	8	2	25%				1.3	Togitu
Grand total	28,221	119	26 (2)	22% (1.7)	41	4	10%	1.1	

Note : Number in parenthesis denote the numbers of non-Japanese encephalitis arbor virus. Isolation efficiency $= \frac{V}{M} \times 1{,}000$

V : No. of pools positive. M : No. of mosquitoes tested for virus isolation.

Japanese encephalitis virus was isolated only from *Culex tritaeniorhynchus* and not from any kind of mosquitoes. The first mosquito from which Japanese encephalitis virus was isolated was collected on May 19, the first day of survey, and the last on July 6 in Aino (Table 5). Therefore, unfortunately we are unable to deduce when the virus first appered in the mosquito prior to May 19. The peak of the infecton of mosquitoes with the virus was in the end of June and its isolation efficiency was as high as 4.7.

Although mosquitoes were still abundant in Aino, there were no more mosquitoes infected with the virus after mid-July. Therefore, we carried out temporary survey in Kaizu and Togitsu in early August in order to clarify whether there were mosqui toes infected with Japanese encephalitis virus other than in Aino area. The survey revealed mosquitoes infected with the virus to be present in both area even though the isolation efficiency was quite low. Upon comparative analysis of isolation of the virus according to the place where the mosquitoes were collected, there was no remarkable difference. The infected mosquitoes were found in all of the livestock pens and the isolation efficiency was higher in cow barns, pig sheds and chicken coops in that order.

Serological characteristics of the isolated Japanese encephalitis virus : All strains of the isolated Japanese encephalitis virus from the mosquitoes were found to have the properties of a wild strain simillar to JaGAr # 01, showing the optimal hemagglutinating potency at pH 6.6—6.8 with one day old chick erythrocytes. As the result of cross hemagglutination inhibition test against JaGAr # 01 strain and Nakayama (NIH) strain, all of the isolated strains turned to be serologically akin to the JaGAr # 01 than the Nakayama.

Discussion

According to MITAMURA et al (1947), many kind of mosquitoes can be infected experimentally with Japanese encephalitis virus in the laboratories. However, it has been revealed that the natural vector mosquitoes of Japanese encephalitis virus are limited as *Culex tritaeniorhynchus* and *Culex pipiens* in Japan. Between those two species, the pricipal vector mosquito in Japan is *Culex tritaeniorhynchus*, since infected *Culex pipiens* is quite small in population and consequently *Culex pipiens* is ecologically not significant (BUESCHER et al., 1959; MATSUYAMA et al., 1960). Our data also revealed that the infected mosquitoes were all *Culex tritaeniorhynchus* among 6 species of the mosquitoes collected.

Our study showed that there were considerable numbers of *Culex tritaeniorhynchus* and some of them had been already infected with Japanese encephalitis virus as early as mid-May whereas up to date, as seen in the reports by BUESCHER et al (1959) and OYA et al (1963), no infected mosquitoes in the Kanto area reported during the early season (April—June) and infection of mosquitoes was considered a regular phenomenon seen in the summer time namely in July to September each year.

Despite the fact that there are many mosquitoes collected in various part of Japan, there has been no single mosquito infected with the virus befor mid-July reported. Therefore, the isolation of the virus from the mosquitoes collected on May 19, 1964 in Aino is the earliest case of infected mosquito with the virus in Japan and Formosa. Although it is not clear whether the infected mosquitoes collectedon may 19, 1964 were eibermated female mosquitoes or new *Culex tritaeniorhynchus* emerged in 1964, judging from thr seasonal prevalence of the mosquito after May 19, 1964, they seem to be new rather than the hibernated ones. It is of interest that the period of the infection of mosquitoes in Aino was over before the population of *Culex tritaeniorhynchus* reached its peak. In the Kanto area, on the contrary, the peak population of the mosquito precedes the period of infection of mosquitoes (Buescher et al., 1959). Therefore further investigation is necessary to see whether this unusual pattern of infection of mosquitoes in Aino is constant or not.

The yearly epidemics of clinically diagnosed human cases of Japanese encephalitis varied in number and time distribution in Nagasaki-ken between 1961 and 1964 (Fig. 4). Namely, the peak of larger epidemics was in the period begining late July to early August, and that of the small epidemics was not conspicuous and the cases were distributed at randomly in August to September. This particular feature of the epidemics is simillar to that of the Kanto area (OYA et al., 1963; SCHERER et al., 1959). From this particular point of view, the epidemics in 1964 in Nagasaki-ken suggested to be that of a large scale but the number of reported cases

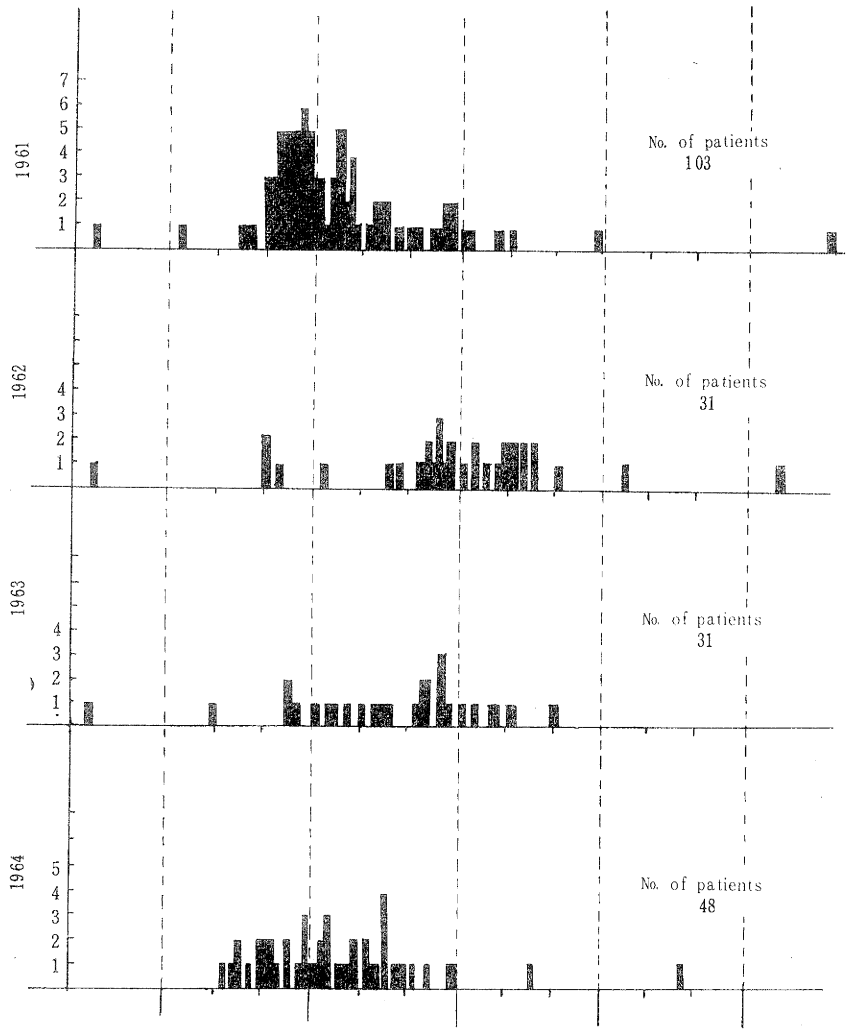

Fig. 4. Number and time distribution of the overt Japsnese encephalitis patients reported in Nagasaki Prefecture in 1961–1964.

constituted the medium sized epidemics.

As for the relationship between the infection of mosquitoes and infection of human, it is said that in general, infection of mosquitoes precedes 2 to 3 weeks infection of human. (BUESCHER and SCHERER., 1959; OYA et al., 1963).

However, the first infection of mosquito in 1964 in Nagasaki was on May 19 and the onset of the first serologicaly verified human case was on July 14. Despite obvious infection among mosquitoes, there were no apparent infection of humans between May 19 and July 14. Following reasons can be considered to explain the time discrepancy between the epidemics among mosquitoes and humans: (1) even though the frequency of detection of the infected mosquitoes was high, the population density of the transmitting mosquitoes had not reached the critical level of transmission of the virus to humans. (2) in the early stage of the season, recently

infected mosquitoes cannot transmit the virus to man, and (3) the phenomenon seen in Aino does not necessarily represent the whole area of Nagasaki-ken.

It has been though previously that the arbor viruses other than Japanese encephalitis virus are quite rare in Japan and not like in tropical area.

However, recently non-Japanese encephalitis arbor viruses have been isolated in the Kanto area. For instace, Negishi virus which was isolated from fatal case of encephalitis in Tokyo in 1948 by ANDO et al (1952) was identified by OKUNO et al (1962) to belong to RSSE family of B-group of arbor virus. Geta virus (Sagiyama virus) which belong to A-group of arbor virus was isolated from mosquitoes in Chiba and Saitama-ken in 1956 and 1957 by SCHERER et al (1962). Later this virus has been detected repeatedly by MATSUYAMA et al (1960) and Hurlbut et al (1964). Akabane virus which was isolated only once in Gumma-ken in 1959 by OYA et al (1961) is a new virus belonging the Shinbu group of arbor virus. When these facts are taken into consideration, it becames apparent that there are arbor viruses other than Japanese encephalitis virus in Japan. As a matter of fact, we also isolated 3 strains of non-Japanese encephalitis viruses from the mosquitoes.

These 3 strains of viruses were isolated from *Culex* when there were no cases of mosquitoes infected with Japanese encephalitis virus. The intracerebral inoculation to mice revealed the viruses to have high pathogenicity to an adult mouse and its incubation period to be short.

These features indicate that these viruses are different from Negishi virus or Geta virus. The identification of those viruses are now in progress and the results will be published in a separate paper upon completion.

Summary

A total of 33,223 female mosquitoes of 6 species (*Culex tritaeniorhynchus*, *Culex pipiens*, *Culex vishnui*, *Culex bitaeniorhynchus*, *Anopheles sinensis*, and *Armigeres subalbatus*) were collected from May 19 to October 30, 1964 in Nagasaki-ken and were submitted for viral isolation. The results are as follows:

(1) 33 strains of arbor viruses were isolated from the 201 pools of mosquitoes. Thirty strains out of the total of 33 strains were identified as Japanese encephalitis virus and the remaining 3 strains were non-Japanese encephalitis viruses.

(2) All of the identified Japan encephalitis virus were isolated from *Culex tritaeniorhynchus*.

(3) In Aino, the first mosquito infected with Japanese encephalitis virus was seen on May 19, 1964 and from that time on the virus was continually isolated from the mosquitoes until July 6, 1964. The rate of infection of mosquitoes with Japanese encephalitis virus was high in late June.

(4) The first mosquito infected with Japanese encephalitis virus was seen 8 weeks prior to the first verified human infection and 11 weeks prior to the peak of the mosquito poqulation. The peak of the infection of mosquitoes was 4 weeks prior to the peak of the infection of human.

(5) Three non-Japanese encephalitis virus strains were isolated from pools of *Culex tritaeniorhynchus* and a mixed pool of *Culex pipiens* and *Culex vishnui*. These mosquitoes were collected during late July to mid-September.

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1964年長崎県における日本脳炎ウィルス感染蚊の疫学的研究. 高橋克巳, 松尾礼三, 熊 正昭, 野口英太郎, 長崎県衛生研究所（所長：高橋克巳博士）

摘　　要

1964年5月19日より，同10月30日迄の間，継続的には県下南高来郡愛野町で，臨時的には西彼杵郡時津町と諫早市貝津で，夜間畜舎（牛舎，豚舎，鶏舎）で捕集した6種（*Culex tritaeniorhyncaus*, *Culex pipiens*, *Culex vishnui*, *Culex biteaniorhynchus*, *Anopheles sinensis*, *Armigeres subatus*）計，33,223匹雌蚊201プールより，哺乳マウス脳内接種法によって，33株の Arbor virus を分離した．そのうち，30株は日本脳炎ウィルスで，総て *Culex tritaeniorhnchus* からのみ分離された．愛野町における最初の日本脳炎ウィルスの分離は，5月19日採取の蚊からで，以後，7月6日迄引続き分離された．分離率が最高を示したのは6月末であった．愛野町において，*Culex tritaeniorhynchus* の発生消長がピークを示した8月上旬，時津町と貝津で臨時に捕集した蚊より，分離率は極めて低いが日本脳炎ウィルスが分離された．他方，1964年の長崎県下の日本脳炎届出患者の発生状況は，例年に比べると中等度の流行と思われ，その発生時期は，7月中旬より8月下旬の間に集中されている．従って，日本脳炎ウィルスの蚊感染と，人の流行との時間的関係は，愛野町における最初の蚊からの日本脳炎ウィルス分離時期（5月19日）が，県下の人の流行開始時期（第1号確認患者の発病は7月14日）に約8週間先行しており，又 *Culex tritaeniorhynchus* の発生消長のピーク（8月上旬）は，人の流行開始時期に約3週間遅れている．この様に1964年の長崎県における日本脳炎ウィルスの蚊感染と人の流行との時間的関係は，従来の他の地方における此の種調査報告に比べると，著るしく特異的であり，又5月19日の時点における日本脳炎ウィルス蚊感染の証明は，我国（沖縄含む）台湾を通じて最も早期であり，共に日本脳炎ウィルスの生態学的に特異なパターンを提示するものである．

3株の非日本脳炎ウィルス分離株のうち，2株は，9月14日採集の *Culex tritaeniorhynchus* より分離され，1株は *Culex pipiens* と *Culex vishnui* の混合プールより分離された．その同定作業は現在進行中である．