

Ecology of Japanese Encephalitis Virus in Okinawa, Japan

I. The investigation on pig and mosquito infection of the virus in Okinawa island from 1966 to 1976

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Abstract : Particular findings of Japanese encephalitis (JE) virus dissemination in nature were investigated in Okinawa island from 1966 to 1976. The first isolation of the virus from pig-sera or *Culex tritaeniorhynchus* mosquitoes as a main vector was usually made at the early part of April or May every year and the virus was disseminated over 100 days in every epidemic season. These findings were not observed anywhere else in Japan including Nagasaki area. Two strains identified as JE virus were isolated from 9 pools of 876 hibernated female mosquitoes of *Culex tritaeniorhynchus* caught in the field by dry ice method from 13 to 15 February 1976 before the appearance of newly emerged vector mosquitoes. The infection of vector mosquitoes and pigs was observed continuously from April to October in 1976. This finding was the second incidence of JE virus isolation from the overwintered female vector mosquitoes following the similar experience in Amami island in 1973. This evidence was of great significance in relation to the overwintering of the virus in Japan.

Attractive investigations on the ecology of Japanese encephalitis (JE) virus have been performed by many workers and the important findings that the mosquitoes of *Culex tritaeniorhynchus* is a main vector of JE virus and pigs serve as an amplifier of the virus in Japan were presented (Mitamura *et al.* 1947; Buescher *et al.* 1959; Scherer *et al.* 1959; Konno *et al.* 1966).

The surveys on the ecology of JE virus in Nagasaki area had been carried out these twelve years since 1964, and the summarized results were presented in serial reports in 1975 (Fukumi *et al.* 1975; Wada *et al.* 1975; Hayashi *et al.* 1975).

Mifune (1965) reported that experimentally infected *Culex tritaeniorhynchus* could carry JE virus through the winter season in Nagasaki and the virus in mosquitoes could be transmitted to susceptible pigs by their biting. The particular attempts to isolate the virus from overwintering mosquitoes of *Culex tritaeniorhynchus* caught in Nagasaki area since 1965. However, so far as the investigation on history of the oviposition of vector mosquitoes was concerned, it was indicated that the possibility of persistence of the virus in vector mosquitoes is extremely low in Nagasaki area (Wada *et al.* 1975).

On the other hand, in Amami island located between the southern part of Kyushu and the main island of Okinawa, it was demonstrated that the infection in vector mosquitoes was persistent in 1973 and interrupted in 1974.

The ecological surveys on JE virus in Okinawa island were performed by U. S. Army researchers, Tigett *et al.* (1950) and Hurlbut *et al.* (1964). However, the virus dissemination in relation to pig and mosquito infection in Okinawa island was not clearly demonstrated. The author has attempted to elucidate the ecology of JE virus in the epidemic and interepidemic seasons in Okinawa island since 1966. This paper describes the particular evidences that the virus dissemination in nature began earlier and in a longer period in Okinawa island than in Nagasaki area, and that the possibility of persistent infection in the overwintered female mosquitoes of *Culex tritaeniorhynchus* in Okinawa island was also demonstrated in 1976.

MATERIALS AND METHODS

Collection of pig-sera : The pig-sera were collected at three slaughter houses, located in the southern, middle and northern parts of Okinawa island, namely, Madambashi (or Ozato), Gushikawa and Nago slaughter houses respectively. Pig-sera were collected every week from January to June and twice a month from July to December. The sera were collected from 5 or 6 months old pigs with identified breeding farms, and were tested for hemagglutination inhibition (HI) antibody and virus isolation (Fig. 1, 2).

Examination of the sera for HI and 2-mercaptoethanol (2ME) sensitive antibody : Antigens used for HI test were Nakayama and JaGar 01 strains which were extracted from the suckling mouse brain with acetone-ether. All of the sera that indicated HI titer over 1:40 were examined for 2ME sensitive antibody. In 1971, the 2ME sensitive antibody in pig-sera were also identified by the treatment with 2ME-Iodoacetoamide instead of 2ME only.

Virus isolation from pig-sera : All the sera that indicated lower HI titer less than 1:10 were examined for the virus isolation through the year by the plaque forming method of chick embryonated (CE) cell culture inoculated with the specimens. The isolates from the plaques were inoculated intracerebrally into suckling mice. The antigens extracted from infected mice brain were tested for HI and neutralization (NT) activity using the antiserum against Nakayama and JaGar 01 strains. Moreover, the viruses in CE cell culture were identified by immunofluorescence technique. In summer season, however, isolation of the virus was attempted by the intracerebral inoculation of the specimens into suckling mice.

Virus isolation from vector mosquitoes: After mosquitoes collected by light traps were fed with 2% sugar solution for several days, they were identified and the attempt to isolate the virus from them was carried out by the method described by Hayashi *et al.* (1965).

Survey of JE virus ecology in Nagasaki area: The results of investigation on the JE virus dissemination in Nagasaki area were kindly presented by the Virus Research Laboratory, Institute for Environmental pollution and Public Health, Nagasaki Prefecture.

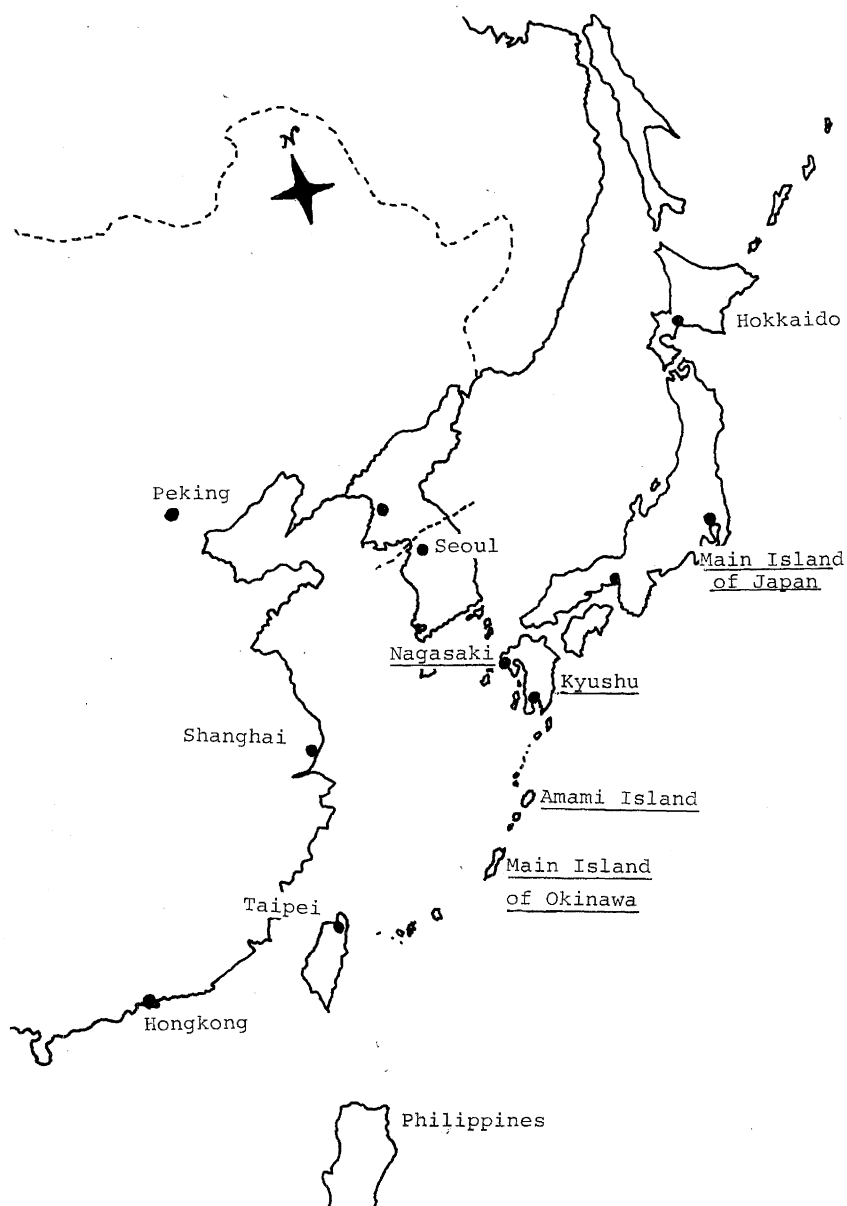


Fig. 1. Map of Japan, Korea and a part of China and USSR



Fig. 2. Map of Okinawa island and slaughter houses located at southern, middle and northern district in the main island of Okinawa.

RESULTS

Virus isolation from pig-sera and vector mosquitoes in Okinawa island

All the sera that indicated HI titer lower than 1:10 were examined for the virus isolation through the survey year since 1969 as described in materials and methods. In addition, the virus isolation from mosquitoes of *Culex tritaeniorhynchus*, particularly the overwintered females in the interepidemic season, was also performed since 1973. It was a noteworthy evidence that the isolates identified as JE virus from pig-sera or vector mosquitoes were obtained from the early part of April or May to the late part of September or the middle part of October in every epidemic season from 1969 to 1975 (Table 1, 2, 3, 4).

In 1976, from 13 to 15 February, 2 strains identified as JE virus were isolated from 9 pools of 876 overwintering female mosquitoes of *Culex tritaeniorhynchus* caught by the dry ice method at Ogimi village near Nago city located at the northern part of Okinawa island (Table 4, 5).

The sequential infections of vector mosquitoes and pigs were detected through the year of 1976 as indicated in Table 4 and 5. It was suggested that the virus might be carried

Table 1. JE virus isolation from vector mosquitoes and pig-sera in Okinawa island from 1969 to 1970

Year	Vector mosquitoes			Pig-sera		
	Month	Places	Isolates	Month	Places	Isolates
1969				Apr. 16	Nago (N)	1
				May 21	Chinen (S)	1
				"	Gushikami (S)	1
				Jun. 11	Naha (S)	1
Total						4
1970				Jun. 2	Nago (N)	1
				"	Motobu (N)	1
				"	Itoman (S)	1
				Jun. 9	Tamashiro (S)	1
				"	Gushikami (S)	1
				Jun. 23	Koza (M)	1
				"	Itoman (S)	1
				"	Haeboru (S)	1
Total						8

Remarks: (N)···Northern area, (M)···Middle area, (S)···Southern area.

Table 2. JE virus isolation from vector mosquitoes and pigs sera in Okinawa island in 1971

Year	Vector mosquitoes			Pig-sera		
	Month	Places	Isolates	Month	Places	Isolates
1971	May 31/ Jun. 1	Nago (N)	1	May 11	Nago (N)	1
				" 18	Nago (N)	1
				" 25	Nago (N)	1
				" 25	Nakijin (N)	1
				" 25	Tomishiro (S)	1
				Jun. 1	Nago (N)	1
				" 1	Nago (N)	1
				" 1	Tomishiro (S)	1
				" 1	Haebaru (S)	1
				" 1	Naha (S)	1
				Jun. 9	Nago (N)	2
				" 9	Nakijin (N)	2
				" 9	Nago (N)	1
				" 9	Nago (N)	1
				" 9	Koza (M)	3
				" 9	Tomishiro (S)	1
				Jun. 15	Nago (N)	1
				" 15	Haneji (N)	1
				" 15	Kunigami (N)	1
				" 15	Koza (M)	1
				" 15	Nakagushuku (M)	1
				" 15	Gushikawa (M)	1
				" 15	Tomishiro (S)	1
				Jun. 22	Nago (N)	2
	" 22	Gushikawa (M)	1			
	" 22	Misato (M)	1			
	" 22	Haebaru (S)	2			
	" 22	Chinen (S)	1			
	Jun. 29	Nago (N)	1			
	" 29	Gushikawa (M)	1			
	" 29	Tamashiro (S)	1			
	Sept. 23/24	Tomishiro (S)	1	Aug. 17	Naha (S)	1
			Sept. 28	Motobu (N)	1	
Total			2			39

in overwintering vector mosquitoes. This particular finding was the second incidence following the case experienced at Setouchi village in Amami island in the interepidemic season in 1973 (Hayashi, *et al.* 1975).

Difference in virus dissemination between Okinawa island and Nagasaki area

The virus isolation from vector mosquitoes or pig-sera was usually performed in the early part of April or May every year in Okinawa island, whereas it was made in the early part of June or August in Nagasaki area. It was also observed that the virus dissemination was noted to begin about 40 to 80 days earlier in Okinawa island than in Nagasaki area as indicated in Table 6. The distinctive intervals of pig infection identified by the

Table 3. JE virus isolation from vector mosquitoes and pig-sera in Okinawa island from 1972 to 1974

Year	Vector mosquitoes			Pig-sera		
	Month	Places	Isolates	Month	Places	Isolates
1972				Jul. 11	Nakijin (N)	1
				" 11	Iotoman (S)	1
				Aug. 2	Gushikawa (S)	1
	Aug. 29/30	Ishikawa (M)	1	" 23	Naha (S)	2
	Sept. 26/27	" (M)	2	Sept. 12	" (S)	2
				" 19	Haeburu (S)	1
	Oct. 3/4	Ishikawa (M)	1	Oct. 17	Chinen (S)	1
	" 20/21	" (M)	1			
" 25/26	Nago (N)	1				
Total			6			9
1973	Jun. 7	Nago (N)	2	Jun. 19	Ishikawa (M)	1
	" 21/22	" (N)	1	" 26	Nago (N)	1
				" 26	Chinen (S)	1
	Jul. 13/14	Nago (N)	1	Jul. 3	Kin (M)	1
				" 21	Naha (S)	1
				" 27	" (S)	1
				Aug. 9	" (S)	2
				" 17	" (S)	1
				" 31	Izena (N)	1
				Sept. 14	Higashi (N)	1
			" 19	Nago (N)	1	
Total			4			12
1974	May 13/14	Nago (N)	1	Jun. 22	Ogimi (N)	2
	Jun. 10/11	" (N)	2	Jnl. 6	Tamashiro (S)	1
				" 13	" (S)	3
				" 27	Itoman (S)	2
				Aug. 10	Naha (S)	1
Total			3			9

Table 4. JE virus isolation from vector mosquitoes and pig-sera in Okinawa island from 1975 to 1976

Year	Vector mosquitoes			Pig-sera		
	Month	Places	Isolates	Month	Places	Isolates
1975	May 12/13	Nago (N)	1	May 30	Ogimi (N)	1
				Jun. 13	Ogimi (N)	1
				" 20	Ogimi (N)	1
				" "	Higashi (N)	1
				Jul. 4	Nago (N)	1
				" "	Yonashiro (M)	1
				" 12	Itoman (S)	1
				" 25	Gushikami (S)	1
				Aug. 1	Naha (S)	1
				" 15	Haebaru (S)	1
				" "	Gushikawa (M)	1
				" 20	Naha (S)	1
				Oct. 16	Ginoza (N)	1
Total			1			13
1976	Feb. 13/15	Ogimi (N)	2			
	Apr. 28/29	" (N)	2			
	May 10/11	" (N)	5	May 25	Nago (N)	1
	Jun. 1/ 2	Nago (N)	3	Jun. 20	Nakijin (N)	2
	" 7/ 8	" (N)	1			
	" 24/25	" (N)	1			
	Jul. 5/ 6	" (N)	2			
				Sept. 17	Nago (N)	1
			" "	Haebaru (S)	1	
Total			16			5

Table 5. JE virus isolation from *Culex tritaeniorhynchus* caught in Okinawa island from February to August 1976

Date	Station	Number of mosquitoes	Number of pools	Number of isolates
Feb. 13/15	Ogimi village	876	9	2
	" "	164	3	0
Mar. 10/11	" "	198	3	0
17/18	" "	169	4	0
27/28	" "	141	3	0
Apr. 11/12	" "	650	7	0
20/21	" "	600	6	0
27/28	" "	1,553	16	2
" "	Nago city	20	1	0
May 4/ 5	Ogimi village	792	8	0
11/12	" "	556	6	5
Jun. 1/ 2	Nago city	212	4	3
7/ 6	" "	151	3	1
17/18	" "	170	4	0
24/25	" "	84	2	1
Jul. 5/ 6	" "	62	2	1
9/20	" "	15	1	1
27/28	" "	41	2	0
Aug. 3/ 4	" "	64	2	0
23/24	" "	23	1	0
Total		6,541	87	16

Remarks : In February and March, the mosquitoes were collected by the method of dry ice evaporation in the field.

detection of 2ME sensitive antibody against JE virus were also observed at the time of the virus isolation from vector mosquitoes or pig-sera as indicated in Table 7. As shown in Table 8, it was a noteworthy evidence that the virus dissemination was detected from April or May to September or October every year in Okinawa island, whereas in Nagasaki area it was usually detected from July to August. After all, the period of virus dissemination in every epidemic season in Okinawa island was over 100 days and that in Nagasaki area was less than 50 days.

Relation between mosquito or pig infection and reported human cases of JE

In Okinawa island, the strains isolated from pig-sera or vector mosquitoes numbered from 14 to 43 from 1971 to 1976 except in 1969 and 1970 when the virus isolation was not made continuously (Table 9). The human incidences, however, decreased in number up to 6 cases or none since 1972 in spite of the numerous isolates and fairly long period of the virus dissemination in the epidemic season.

In contrast, in Nagasaki area, the number of isolated strains varied from 32 to 71 during 1965 to 1971, while the virus dissemination in nature varied from 35 days in 1968 to 100 days in 1965. The strains ranging in number from 2 to 19 were isolated from 1972 to 1976. A large number of human JE cases occurred in 1965 and 1966. However,

Table 6. Distinctive time of the first JE virus isolation in the field in Okinawa island and Nagasaki area from 1966 to 1976

Year	Area	Virus isolation	Intervals of virus isolation between Nagasaki and Okinawa
1966	Okinawa	n.d.	—
	Nagasaki	Jun. 21	
1967	Okinawa	n.d.	—
	Nagasaki	Jun. 6	
1968	Okinawa	n.d.	—
	Nagasaki	Jul. 18	
1969	Okinawa	Apr. 16	82
	Nagasaki	Jul. 9	
1970	Okinawa	Jun. 2	43
	Nagasaki	Jul. 15	
1971	Okinawa	May 11	62
	Nagasaki	Jul. 12	
1972	Okinawa	Jul. 11	40
	Nagasaki	Aug. 16	
1973	Okinawa	Jun. 6	36
	Nagasaki	Jul. 9	
1974	Okinawa	May 13	77
	Nagasaki	Jul. 29	
1975	Okinawa	May 12/13	45
	Nagasaki	Jul. 14	
1976	Okinawa	Feb. 13/15	125
	Nagasaki	Jul. 21	

Remarks: n.d. not done.

Table 7. Intervals of the first detection of 2ME sensitive HI antibody in pig-sera between Okinawa island and Nagasaki area from 1966 to 1976

Year	Area	Date of detection of 2ME sensitive HI antibody	Intervals of HI antibody rising between Nagasaki and Okinawa
1966	Okinawa	Apr. 11	84
	Nagasaki	Jul. 4	
1967	Okinawa	May 2	51
	Nagasaki	Jun. 22	
1968	Okinawa	May 15	66
	Nagasaki	Jul. 20	
1969	Okinawa	May 7	78
	Nagasaki	Jul. 24	
1970	Okinawa	Jun. 9	59
	Nagasaki	Aug. 7	
1971	Okinawa	May 4	63
	Nagasaki	Jul. 6	
1972	Okinawa	Jun. 27	40
	Nagasaki	Aug. 5	
1973	Okinawa	May 23	63
	Nagasaki	Jul. 24	
1974	Okinawa	May 24	78
	Nagasaki	Aug. 6	
1975	Okinawa	Jun. 13	34
	Nagasaki	Jul. 15	
1976	Okinawa	May 4	70
	Nagasaki	Jul. 13	

Table 8. Difference in JE virus dissemination in the field between Okinawa island and Nagasaki area from 1969 to 1976

Year	Okinawa island		Nagasaki area*	
	Virus isolation	Period (days)	Virus isolation	Period (days)
1969	Apr. 16 — n.d.	—	Jul. 9 — Aug. 28	51
1970	Jun. 2 — Oct. 17	138	Jul. 15 — Aug. 31	48
1971	May 11 — Sept. 28	141	Jul. 12 — Aug. 24	44
1972	Jul. 11 — Oct. 25/26	108	Aug. 16 — Sept. 9	25
1973	Jun. 6/7 — Sept. 19	107	Jul. 9 — Aug. 13	36
1974	May 13/14 — Aug. 10	90	Jul. 29 — Aug. 13	15
1975	May 12/13 — Aug. 20	100	Jul. 14 — Aug. 25	43
1976	Feb. 13/15 — Sept. 17	217	Jul. 21 — Aug. 9	20

Remarks : The data in the column of Nagasaki area (*) was kindly presented by the Institute for the Environmental Pollution and Public Health of Nagasaki Prefecture.

the number of cases decreased gradually from 1968 and only several cases a year have been reported since 1971 (Table 10).

It must be noted, however, that the number of isolates in the epidemic season as described above (Table 9 to 10) does not present the real aspect of pig and mosquito infection. After all, the period of virus dissemination and the number of strains isolated in every epidemic season both in Okinawa island and Nagasaki area were not considered to be correlated with the size of human epidemic.

Table 9. JE virus isolation from pig sera and *Culex tritaeniorhynchus* mosquitoes and human encephalitis cases in the main island of Okinawa from 1966 to 1976

Year	JE virus isolation		Reported human cases	
	Isolation period (days)	Number of isolates	Number of cases	Periods
1969	Apr. 16 - Jun. 11 - n.d.	4	22	Mar. 25 - Oct. 15
1970	Jun. 2 - Oct. 17 (138)	8	19	May 22 - Nov. 2
1971	May 11 - Sept. 28 (141)	41,(2)	36	May 17 - Sept. 22
1972	Jul. 11 - Oct. 25/26 (108)	15,(6)	4	Jun. 1 - Nov. 4
1973	Jun. 6/7 - Sept. 19 (107)	16,(4)	9	Mar. 15 - Dec. 17
1974	May 13/14 - Aug. 10 (90)	12,(3)	0	-
1975	May 12/13 - Aug. 12/13 (100)	13,(1)	3	Jul. 31 - Aug. 23
1976	Feb. 13/15 - Sept. 17 (217)	21,(16)	0	-

Remarks : (1) n.d.not done (2) The number of isolates and the number in parentheses mean the isolates from pig sera and *Culex tritaeniorhynchus* mosquitoes respectively

Table 10. JE virus isolation from *Culex tritaeniorhynchus* mosquitoes and human encephalitis cases in Nagasaki from 1964 to 1975

Year	JE virus isolation from <i>C. tritaeniorhynchus</i>		Human encephalitis cases	
	Term of virus isolation	Number of virus isolates	Number of reported cases	Periods
1964	Jun. 8 - Aug. 7 (61)	19	45	Jul. 3 - Sept. 10
1965	May 30 - Sept. 6 (100)	47	68	Jul. 5 - Oct. 23
1966	Jun. 24 - Aug. 27 (65)	41	127	Jul. 2 - Nov. 26
1967	Jun. 6 - Jul. 26 (51)	43	43	Jun. 18 - Sept. 7
1968	Jul. 18 - Aug. 21 (35)	39	20	Jul. 5 - Sept. 30
1969	Jul. 9 - Aug. 28 (51)	33	19	Jul. 22 - Sept. 26
1970	Jul. 15 - Aug. 31 (48)	50	17	Jul. 23 - Sept. 18
1971	Jul. 12 - Aug. 24 (44)	32	3	Aug. 8 - Sept. 15
1972	Aug. 16 - Sept. 9 (25)	2	1	Aug. 10
1973	Jul. 9 - Aug. 13 (36)	19	6	Jul. 30 - Sept. 4
1974	Jul. 29 - Aug. 12 (15)	3	2	Jul. 21, Jul. 24
1975	Jul. 14 - Aug. 25 (43)	16	2	Jul. 19, Aug. 4
1976	Jul. 21 - Aug. 9 (20)	7		

Remarks : The data from 1967 to 1976 were kindly presented by the Institute for Environmental Pollution and Public Health of Nagasaki Prefecture.

Table 11. Isolation of Japanese encephalitis virus from *Culex tritaeniorhynchus* in the main island of Okinawa from 1972 to 1976

Year	1972			1973			1974			1975			1976		
Month	No. of Mosq. tested	No. of Pools	No. of virus isolated	No. of Mosq. tested	No. of Pools	No. of virus isolated	No. of Mosq. tested	No. of Pools	No. of virus isolated	No. of Mosq. tested	No. of Pools	No. of virus isolated	No. of Mosq. tested	No. of Pools	No. of virus isolated
January	n.d.			139	3	0	292	5	0	n.d.			n.d.		
February	"			1,059	13	0	841	12	0	12	1	0	1,040	12	2(N)
March	"			n.d.			415	6	0	494	13	0	508	10	0
April	"			967	10	0	186	2	0	570	14	0	2,823	30	2(N)
May	"			405	6	0	375	6	1(N)	618	15	1(N)	1,348	14	5(N)
June	"			817	10	3(N)	187	3	2(N)	n.d.			617	13	5(N)
July	408	5	0	574	7	1(N)	n.d.			"			118	5	2(N)
August	1,954	24	1(M)	487	5	0	"			"			87	3	0
September	4,525	51	2(N)	424	5	0	"			"			n.d.		
October	1,032	16	3(M,N)	451	6	0	20	1	0	"			n.d.		
November	399	7	0	n.d.			n.d.			"			n.d.		
December	n.d.			"			"			"			n.d.		
Total	8,318	103	6	5,313	65	4	2,316	35	3	1,694	41	1	6,541	87	16

Remarks: (N)...Northern area, (M)...Middle area, (S)...Southern area.

DISCUSSION

The ecological survey on JE virus in Okinawa island was carried out only by Tigertt *et al.* (1950) and Hurlbut *et al.* (1965). The attempt to isolate the virus from 317 pools of 46, 788 mosquitoes of *Culex tritaeniorhynchus* caught from February to November 1960 was carried out by Hurlbut *et al.* (1964), and 5 strains in August, 32 strains in September and 5 strains in October were isolated from vector mosquitoes. The author has investigated the virus dissemination in the epidemic season and overwintering of the virus in the interepidemic season in Okinawa island, since 1966. The examination for only HI antibody in pig-sera was made from 1966 to 1968, and in addition the virus isolation from all of pig-sera indicating the titer lower than 1:10 and from vector mosquitoes was performed since 1969 and 1973 respectively. So far as the results obtained from 1973 to 1975 were concerned, the virus isolation from vector mosquitoes was made earlier than that from pig-sera collected in the limited same survey area in Okinawa island. It was also a noticeable fact that the starting time and the period of virus dissemination in Okinawa island were earlier and longer than that in Nagasaki area (Table 8, 9, 10). These findings of the virus dissemination investigated in Okinawa island were never seen in the main island of Japan. Attempts to isolate the virus from 16 pools of 1,198 mosquitoes in 1973, 25 pools of 1,548 mosquitoes in 1974 and 14 pools of 506 mosquitoes caught from January to March in every year were unsuccessful (Table 11). It was, however, a very important evidence for the consideration about the overwintering of the virus in Okinawa island that 2 strains of JE virus were isolated from overwintering female vector mosquitoes caught in the

middle part of February 1976 and the sequential infections of vector mosquitoes and pigs were observed through the year. It has been suggested that the virus might have been carried with the overwintering vector mosquitoes in favourable condition in the interepidemic season in 1976. Such a persistence of the virus in overwintering mosquitoes of *Culex tritaeniorhynchus* and the maintenance of the cycle of the virus between vector mosquitoes and pigs through the year was the second incidence since the similar finding was investigated in 1973 in Setouchi village in Amami island as reported by Hayashi *et al.* (1975). After all, the overwintering of JE virus with vector mosquitoes was demonstrated in certain favourable condition in the southern part of Japan such as Okinawa and Amami islands.

ACKNOWLEDGEMENT

Author has been greatly indebted to the Virological Laboratory of the Institute for Environmental Pollution and Public Health, Nagasaki Prefecture for the kind cooperation of comparative investigation on JE virus ecology. Author also express sincere thanks to Dr. Tetsu Shimabukuro, chief of Diagnostic Section of Animal Disease, Animal Health Center, Okinawa Prefecture for the kind supply of suckling mice, and to Mr. Kunio Nakachi, Mr. Takao Kishimoto, Mrs. Yoshiko Higa, Mrs. Kazuko Shimojana, staffs of our Institute, for the kind cooperation of mosquito collection and classification. Author would like to thank Prof. Kaoru Hayashi for his continuous encouragement throughout this study.

REFERENCE

- 1) Buescher, L., Scherer, W. F., Rosenberg, M. Z., Gresser, I., Hardy, J. L. & Bullock, H. R. (1959) : Ecologic studies of Japanese encephalitis virus in Japan. 2. Mosquito infection. *Amer. J. Trop. Med. Hyg.*, 651-664.
- 2) Fukumi, H., Hayashi, K., Mifune, K., Shichijo, A., Matsuo, S., Omori, N., Wada, Y. & Mori, A. (1975) : Ecology of Japanese encephalitis virus in Japan. I. Mosquito and pig infection with the virus in relation to human incidences. *Trop. Med.*, 17, 97-110.
- 3) Hayashi, K., Mifune, K., Schichijo, A., Suzuki, H., Matsuo, S., Makino, Y., Akashi, M., Wada, Y., Oda, T., Mogi, M. & Mori, A. (1975) : Ecology of Japanese encephalitis virus in Japan. III. The results of investigation in Amami island, southern part of Japan, from 1973 to 1975. *Trop. Med.*, 17, 129-142.
- 4) Hayashi, K., Mifune, K., Motomura, I., Matsuo, S., Kawazoe, H. & Futatsuki, K. (1965) : Isolation of Japanese encephalitis virus from mosquitoes collected in Omura district, Nagasaki prefecture, Japan in 1964. *Endem. Dis. Bull. Nagasaki Univ.*, 7, 155-164.
- 5) Hurlbut, H. S. & Nibley, C. (1964) : Virus isolations from mosquitoes in Okinawa. *J. Med. Ent.*, 1, 78-83.
- 6) Konno, J., Endo, K., Agatsuma, H. & Ishida, N. (1966) : Cyclic outbreaks of Japanese encephalitis among pigs and humans. *Amer. J. Epid.*, 84, 292-300.
- 7) Mifune, K. (1965) : Transmission of Japanese encephalitis virus to susceptible pigs by mosquitoes of *Culex tritaeniorhynchus* after experimental hibernation. *Endem. Dis. Bull. Nagasaki Univ.*, 7,

178-191.

- 8) Mitamura, T. & Kitaoka, M. (1947) : On the epidemiology of epidemic encephalitis. 12th J. Japanese medical society. 47-68 (in Japanese) quoted in medical microbiology Ed. Fukumi, H. et al. Igaku-shoin.
- 9) Scherer, W. F., Moyer, J., Izumi, T., Gresser, I. & McCown, J. (1958) : Ecologic studies of Japanese encephalitis virus in Japan. 6. Swine infection. Amer. J. Trop. Med. Hyg., 8, 698-706.
- 10) Tigertt, W. D. & Hammon, W. M. (1950) : Japanese B encephalitis : A complete review of experience in Okinawa, 1945-1949. Amer. J. Trop. Med. Hyd., 30, 689-722.
- 11) Wada, Y., Oda, T., Mogi, M., Mori, A., Omori, N., Fukumi, H., Hayashi, K., Mifune, K., Schichijo, A. & Matsuo, S. (1975) : Ecology of Japanese encephalitis virus in Japan. II. The population of vector mosquitoes and the epidemic of Japanese encephalitis. Trop. Med., 17, 111-127.

沖縄における日本脳炎ウイルスの生態の研究

1. 沖縄本土におけるコガタアカイエカ及び豚の日本脳炎ウイルス感染の実態

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沖縄本島における日本脳炎(日脳)ウイルスの疫学的調査は Tigertt *et al.* (1950)及び Hurlbut *et al.* (1964) によって行われているにすぎない。同氏等によると 捕集蚊からウイルスが分離されるのは8月初旬から10月初旬までであったと述べている。著者は1966年以後、沖縄の日脳ウイルスの生態の研究に着手し、特に1969年以後は血球凝集抑制(HI)抗体1:10以下の豚血清全例について、1972年以後は野外蚊からのウイルス分離をも平行して行った。また、沖縄本土におけるウイルス汚染の状況を長崎地方のそれと比較し沖縄本土のウイルス撒布の特異性を検討した。沖縄本土では蚊及び豚の日脳ウイルス感染は例年4月ないし5月に開始し、それと平行して豚血清中の2-メルカプトエタノール(2ME)感受性抗体も検出された。自然界でのウイルス汚染は例年100日以上、長いときは186日にも達し、かつウイルス汚染の開始は長崎地方のそれより40日以上90日も早い。このような日脳ウイルスの汚染は日本本土では認められない現象である。1976年2月、876個体9プールの越冬コガタアカイエカから2株の日脳ウイルスが分離され、引続いて9月まで豚血清からウイルスの分離が可能であった。豚血清中の2ME感受性抗体は4月から10月まで検出され、年間ウイルス撒布が持続していたことを示した。沖縄では条件がよければ、1976年のように、蚊-ブタ-蚊のウイルス生存環が保たれ、特に蚊によるウイルスの越冬が可能であることを物語っている。そして、このような事実は1973年2月越冬蚊から日脳ウイルスが分離された奄美大島瀬戸内における調査成績に次ぐもので、日脳ウイルスの越冬の問題に重要な資料となるものである。