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Ecology of Japanese Encephalitis Virus in Japan

III. The results of investigation in Amami island, southern part of Japan, from 1973 to 1975

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ABSTRACT : Characteristics of the ecology of Japanese encephalitis (JE) virus dissemination were investigated in Amami island located between the southern part of Kyushu and the main island of Okinawa. Four strains identified as JE virus were isolated from 8 pools of 1083 hibernated female mosquitoes of *Culex tritaeniorhynchus* caught in the field from 3rd to 18th February, 1973, before the appearance of newly emerged vector mosquitoes. This finding suggested the overwintering of the virus in the vector mosquitoes in survey areas. The virus dissemination in the survey area in 1973 was observed through the year in connection with the cycle of vector mosquitoes and pigs infection. In 1974, however, the virus isolation from vector mosquitoes was performed in July. This evidence indicated the interruption of the persistence of the virus in vector mosquitoes and the virus might be carried into the survey area in Amami island. These findings were of great significance in connection with the problems on the overwintering of JE virus in Japan. In the midnight on 24th to the very early morning on 25th July, 1973, mosquitoes of *Culex tritaeniorhynchus* were captured with the light traps set up on the ship sailing between the south part of Kyushu (Kagoshima) and Amami island.

This finding suggest that vector mosquitoes might be transported with the wind over the ocean. In accordance with these evidence, the attempt to disperse mosquitoes of *Culex tritaeniorhynchus* experimentally labeled with dyes from Amami island to the southern part of Kyushu (Kagoshima) was made under the selected condition of the weather in the end part of July, 1975. It was, however, unsuccessful with hindering of occassionally happened typhoon.

Serial investigations on the ecology of JE virus in Nagasaki area were carried out during these ten years since 1964. Although the virus dissemination in nature was observed in the long duration of one month or more in each year from 1964 to 1968, it had been shortened up to two weeks after 1969. In spite of the fact that the virus dissemination in nature had been decreased and shortened in duration recently, it was often found that the infection of vector mosquitoes and pigs after the middle part of July since 1969 until 1975.

On the other hand, the attempt to isolate JE virus from 80153 hibernated female mosquitoes of *Culex tritaeniorhynchus* caught in early spring since 1965 had been unsuccessful. It was considered that there may be unique circumstances for the virus dissemination in nature particularly for the overwintering of the virus in Nagasaki area located in the temperate zone. However, the favourable situation for the transmission cycle and the interepidemic persistence of the virus might vary by area among the temperate, subtropical and tropical zone.

Taking into consideration about these geographical situation, the investigation on the ecology of JE virus in Amami island has been carried out since 1973. The unique dissemination of the virus in survey area in Amami island has been observed and the results will be presented in this paper. Furthermore, it will be described that the attempt to disperse the labeled mosquitoes with dyes towards the north "it means the southern part of Kyushu" from the Amami island under the selected weather at the end part of July, 1975.

MATERIALS AND METHODS

Places and methods for the mosquito collection:

Although five places were selected for the mosquito collection at the beginning period of the survey, the continuous investigations have been made at least at three places as seen in Fig. 1 and Fig. 2. The mosquitoes were caught usually by light traps set up in the cowsheds and pigsheds and the dry ice method (Omori et al., 1965) was also applied in interepidemic season.

Collection of sera of pigs, hens and other animals:

The pigsera were collected through the year from indigenous pigs except imported pigs from other places of Japan. During interepidemic season, the sera from hens, rats, snakes and wild boars were collected and examined the hemagglutination inhibiton antibody.

Virus isolation from mosquitoes and identification of the virus isolated:

At the beginning period of the study, the mosquitoes caught in the survey area were transported by airlift to the laboratory of the Department of Virology, Institute for Tropical Medicine from the survey station in Koniya village located in the southern part of Amami island (Fig. 2). The mosquitoes transported were anaesthetized with carbon dioxide and identified. On the other hand, in the later part of the study, after the mosquitoes collected were identified at the survey station, they were stored in tubes kept in dry-ice-acetone and transported by airlift to the laboratory in the Institute. The virus

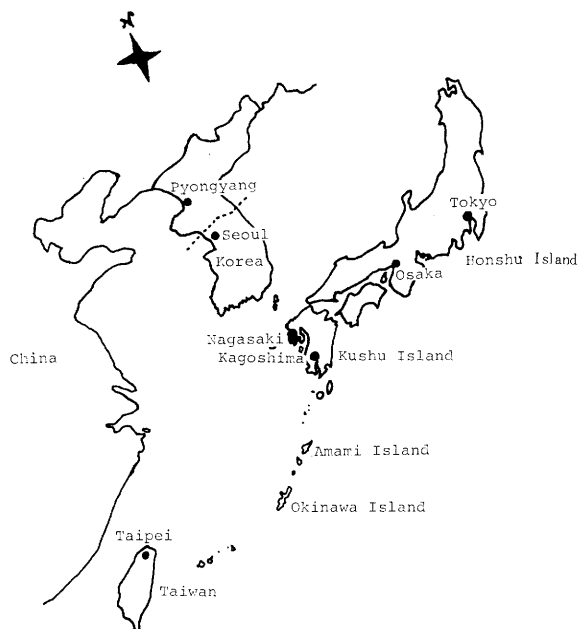


Fig. 1. Map of Japan, Korea and a part of China

isolation from mosquitoes and the identification of the virus isolated were made as described in the previous paper (Hayashi et al., 1965).

On the other hand, the mosquitoes of *Culex tritaeniorhynchus* collected particularly in interepidemic season were also examined the history of oviposition and the development of follicles.

Virus isolation from nymph, larva and adults of ticks:

Amblyomma testudinarium, *Haemaphysalis formosensis* and *H. hystricis* caught on the foot of mountains were examined for the virus isolation in March, 1973. One ml of diluent of phosphate buffered saline contained 0.5% bovine albumin was added to a tick and homogenized in ice bath. After centrifugation at 10,000 rpm for 20 minutes, the supernatants were inoculated into suckling mouse intracerebrally by the same method as in case of the virus isolation from vector mosquitoes.

Hemagglutination inhibition (HI) test:

HI antibody, particular 2-mercaptoethanol (2 ME) sensitive antibody against JE virus in pig-sera was examined by the same method as described in previous papers (Hayashi et al., 1965, 1968). In case of the examination of hen-sera, the elimination of the inhibitor was carried out as follows: 0.2 ml of protamine sulfate was added to 0.1 ml of serum to make a final concentration of 1 mg of protamine sulfate. After the mixture was shaken with cold acetone, the acetone was evaporated in the desiccator. The dried serum powder was resuspended with 0.1 ml of Borate buffer at pH 9.0 and kept at 4°C overnight. The treated serum was adsorbed with chicken red cells and spun down. The supernatant was used for HI test at a initial dilution of 1:10.

RESULTS

Virus isolation from Culex tritaeniorhynchus:

The results of virus isolation from vector mosquitoes through the year of 1973 were



Fig. 2. Map of Amami island and survey places.

Table 1. Isolation of JE virus from *Culex tritaeniorhynchus* in Amami island in 1973

Date of collection	No. of mosq.	No. of pools	No. of isolates
1973 Feb.	E	129	2
	M	954	6
	L	339	2
Mar.	E	—	—
	M	336	7
	L	73	3
Apr. E-L	131	5	0
May	E	266	9
	M	82	3
	L	208	4
Jun.	E	—	—
	M	361	4
	L	43	4
Jul.	E	—	—
	M	897	3
	L	1,043	7
Aug.	E	789	4
	M	227	2
	L	410	5
Sep.	E	593	4
	M	1,234	8
	L	671	9
Oct.	E	1,281	13
	M	—	—
	L	121	3
Nov.	E	44	1
	M	33	1
	L	—	—
Total	10,265	109	13

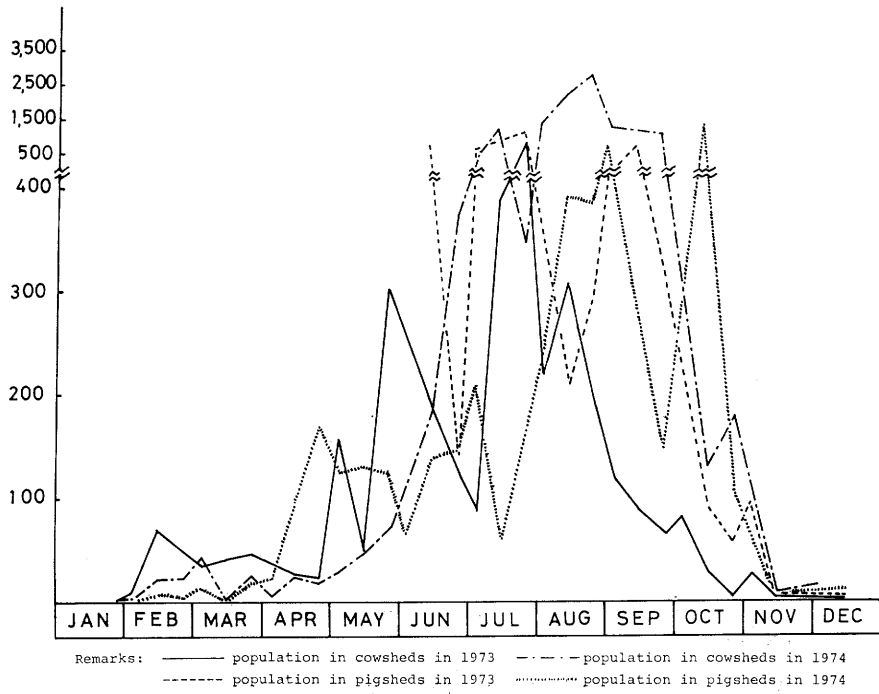


Fig. 3. Seasonal prevalence of *Culex tritaeniorhynchus* in 1973 and 1974.

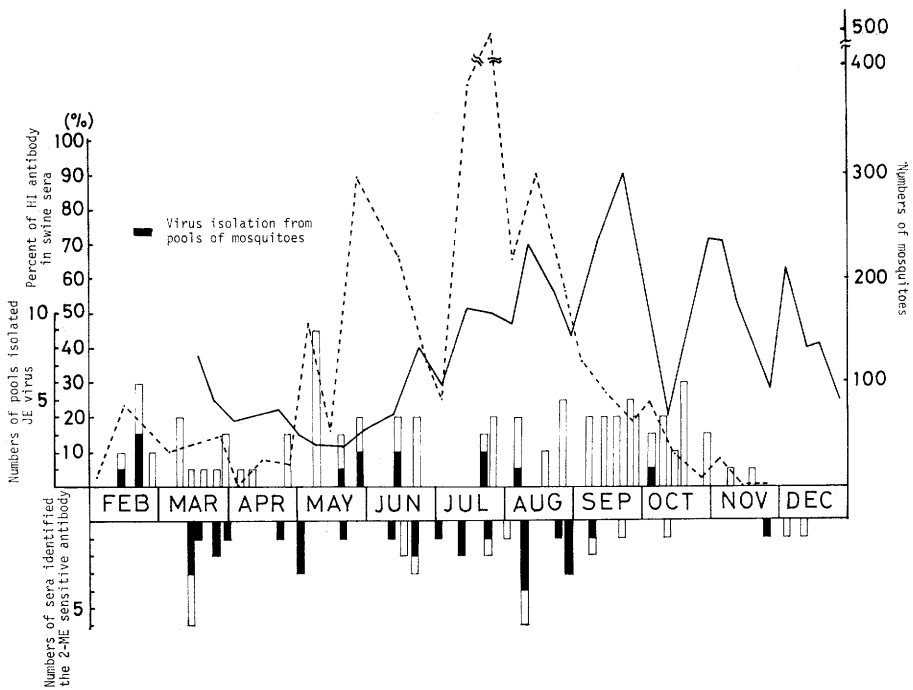


Fig. 4. Correlation between the infection of *Culex tritaeniorhynchus* mosquitoes and swine with Japanese encephalitis virus and seasonal prevalence of the mosquitoes in Amami island in 1973.

shown in Table 1 and Fig. 4. There were 4 strains isolated from 8 pools of 1083 hibernated female mosquitoes of *Culex tritaeniorhynchus* caught in the survey area from 3rd to 18th of February. These strains were identified as JE virus. The sequential infection of vector

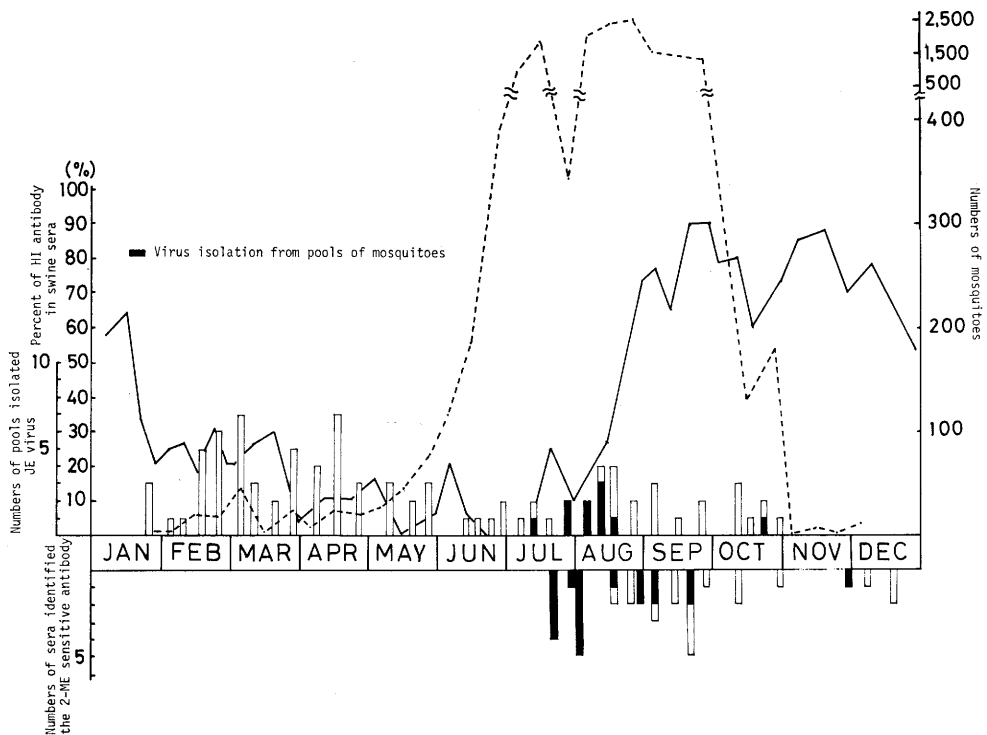


Fig. 5. Correlation between the infection of *Culex tritaeniorhynchus* mosquitoes and swine with Japanese encephalitis virus and seasonal prevalence of the mosquitoes in Amami island in 1974.

mosquitoes were detected through the year of 1973. It suggested that the virus might persist in overwintering vector mosquitoes of *Culex tritaeniorhynchus*.

In 1974, the virus isolation from vector mosquitoes was made at first on the middle part of July. However, it was noted that the mosquito infection was detected occasionally up to the end part of October (Table 2 and Fig. 5). In 1975, the mosquito infection was not demonstrated until the early part of June (Table 3).

These findings suggested that the cycle of JE virus infection between mosquitoes and pigs might be interrupted in winter of 1974 and 1975 (Table 2, Table 3, Fig. 5).

Table 2. Isolation of JE virus from *Culex tritaeniorhynchus* in Amami island in 1974

Date of collection	No. of mosq.	No. of pools	No. of isolates
Jan. E	—	—	—
M	—	—	—
L	25	3	0
Feb. E	54	2	0
M	335	5	0
L	572	6	0
Mar. E	838	10	0
M	35	2	0
L	253	5	0
Apr. E	366	4	0
M	935	7	0
L	538	3	0
May E	579	3	0
M	333	2	0
L	460	3	0
Jun. E	—	—	—
M	457	2	0
L	756	3	0
Jul. E	719	3	1
M	157	1	0
L	319	2	2
Aug. E	1,352	6	5
M	764	4	1
L	417	2	0
Sep. E	765	4	0
M	—	—	—
L	499	2	0
Oct. E	497	3	0
M	358	3	1
L	104	1	0
Nov. E-L	26	1	0
Dec. E-L	22	1	0
Total	12,535	93	10

Table 3. Isolation of JE virus from *Culex tritaeniorhynchus* in Amami island in 1975

Date of collection	No. of mosq.	No. of pools	No. of isolates
Jan. L — Feb. E	23	1	0
Apr. E	—	—	—
M	180	4	0
L	186	2	0
May E	558	4	0
M	129	2	0
L	—	—	—
Jun. E	215	1	0
Total	1,091	14	0

Age-group of the female mosquitoes of Culex tritaeniorhynchus:

The female mosquitoes of *Culex tritaeniorhynchus* caught in the survey area during from the early part of March to the middle part of April in 1974 were examined the status of oviposition and the development of their follicles.

In total, 24 and 95 of 119 female mosquitoes were found to be parous and nulliparous respectively. On the other hand, most of these female mosquitoes were situated in the development of lb stage.

These finding suggested that the resting female vector mosquitoes might have recovered their feeding activity when the weather became warmer by day even though it was the winter period (Table 4).

Table 4. Age-group of *Culex tritaeniorhynchus* caught in Amami island in 1974

Date of collection	Number of mosquitoes dissected	Number parous	Number nulliparous	No. mosquitoes with each follicular stage
March 5, 6	25	5	20	24(Ib) 1 (unidentified)
7, 9	20	4	16	15(Ib) 2(I-II) 2(IIa) 1 (unidentified)
22,23,24	19	7	12	14(Ib) 3(IIa) 2 (unidentified)
25,26	10	3	7	8(Ib) 2(IIa)
31	8	0	8	8(Ib)
April 1, 3	5	1	4	5(Ib)
9,10	20	1	19	20(Ib)
11,12	12	3	9	12(Ib)
Total	119	24	95	106(Ib) 2(I-II) 2(IIa) 5(IIb) 4 (unidentified)

Seasonal prevalence of vector mosquitoes in 1973 and 1974:

Seasonal prevalence of *Culex tritaeniorhynchus* female mosquitoes collected by light traps in pigsheds and cowsheds in 1973 and 1974 are illustrated in Fig.3. The fairly small number of vector mosquitoes was found from the middle part of November to the middle part of next March in both years. Although it was interepidemic season, the hibernated female vector mosquitoes gained their gonoactivity when the weather became warmer as described above. The newly emerged mosquitoes were detected usually in the end part of March every year, and the population of them increased rapidly from the middle or the end part of April.

Virus isolation from Culex pseudovishnui:

Attempt to isolate the virus from, in total, 1,281 mosquitoes of *Culex pseudovishnui* collected in hensheds by light traps during the end part of September in 1973 to the middle part of May in 1975 was made. It was, however, noted that no virus could be isolated at all.

HI antibody in pigsera:

With reference to the results of JE virus isolation from vector mosquitoes through the year from 1973 to 1975, it was recognized the remarkable findings that the detection of HI antibody particularly 2 ME sensitive antibody in indigenous pigsera was made closely in parallel with the mosquito infections. Although the continuous infection of pigs was observed through the year in 1973, it was interrupted in winter period in 1974. The

Table 5. HI antibody against JE virus in pig-sera in 1973

Date of collection	No. of positive		Percent of positive	No. of sera 2-ME sensitive
	No. of sera tested			
Mar. M	30/78		38.5	7/30
	14/55		25.5	2/14
Mar. L—Apr. E	12/62		19.4	1/12
Apr. M	5/23		21.7	1/5
May E	5/37		13.5	3/5
	2/16		12.5	0/2
	3/18		16.7	0/3
Jun. E	—		—	—
	7/34		20.6	1/7
	8/20		40.0	3/8
Jul. E	5/17		29.4	1/5
	10/19		52.6	2/10
	7/14		50.0	2/7
Aug. E	21/35		60.0	7/21
	9/16		56.3	1/9
	8/18		44.4	3/8
Sep. E	13/18		72.2	2/13
	10/11		90.9	1/10
	—		—	—
Oct. E	11/17		64.7	0/11
	3/15		20.0	1/3
	18/29		62.1	0/18
Nov. E	10/14		71.4	0/10
	15/33		45.5	0/15
	5/18		27.8	1/5
Dec. E	15/27		55.6	2/15
	8/19		42.1	0/8
	5/19		26.3	0/5
Total	259/672		38.5	41/259

Table 6. HI antibody against JE virus in pig-sera in 1974

Date of collection	No. of positive		Percent of positive	No. of sera 2-ME sensitive
	No. of sera tested			
Jan.	E	7/12	58.3	0/7
	M	9/14	64.3	0/9
	L	9/29	31.0	0/9
Feb.	E	2/8	25.0	0/2
	M	9/39	23.1	0/9
	L	10/40	25.0	0/10
Mar.	E	9/42	21.4	0/9
	M	17/81	21.0	0/17
	L	15/80	18.8	0/15
Apr.	E	6/39	15.4	0/6
	M	5/36	13.9	0/5
	L	1/17	5.9	0/1
May	E	6/38	15.8	0/6
	M	0/19	0	0
	L	2/33	6.1	0/2
Jun.	E	4/19	21.1	0/4
	M	1/36	2.8	0/1
	L	0/20	0	0
Jul.	E	0/19	0	0
	M	5/35	14.3	4/5
	L	1/16	6.3	1/1
Aug.	E	5/35	14.3	5/5
	M	17/41	41.5	4/17
	L	11/15	73.3	2/11
Sep.	E	13/17	76.5	3/13
	M	29/37	78.4	7/29
	L	18/20	90.0	1/18
Oct.	E	31/39	79.5	2/31
	M	12/30	60.0	0/12
	L	14/19	73.7	1/14
Nov.	E	17/20	85.0	0/17
	M	15/17	88.2	0/15
	L	14/20	70.0	1/14
Dec.	E	14/18	77.8	1/14
	M	9/19	47.4	2/9
	L	12/22	54.5	0/12
Total	349/1,041		37.8	34/349

pig infection was detected in the middle part of July and continued until the middle part of December, 1974. However, it was interrupted again in winter period as seen in Table 5, Table 6 and Table 7.

HI antibody in sera of hens and other animals:

Four of 130 sera of hens collected from 10th October, 1973 to 16th April, 1974 in interepidemic season, one sheep serum and 2 of 11 sera of wild boars collected from 21st

Table 7. HI antibody against JE virus in pig-sera in 1975

Date of collection	No. of positive		Percent of positive	No. of sera 2-ME sensitive
	No. of sera tested			
Jan. E M L	6/15		40.0	0/6
	11/17		64.7	0/11
	7/17		41.2	0/7
Feb. E M L	11/21		52.4	0/11
	9/16		56.3	0/9
	5/16		31.3	0/5
Mar. E M L	0/13		0	0
	9/16		56.3	0/9
	2/14		14.3	0/2
Apr. E M L	4/12		33.3	0/4
	4/15		26.7	0/4
	2/12		16.7	0/2
May E M L	1/16		6.3	0/1
	1/11		9.1	0/1
	4/14		28.6	0/4
Jun. E M L	4/15		26.7	0/4
	1/16		6.3	0/1
	1/15		6.7	0/1
Jul. E M	1/20		5.0	0/1
	2/11		18.2	0/2
Total	84/319		26.3	0/85

January to 26th February 1974 were indicated to have lower titer (1 : 20-40) of HI antibodies. However, 127 of 130 sera of hens and 59 of 124 sera of wild boars, wild rats and *Trimeresurus flavoviridis* were not demonstrated the HI antibodies during the interepidemic season.

Attempt to isolate the virus from ticks:

One hundred and sixtyfour ticks and 30 lice infested on wild boars and pigs respectively collected on 22nd and 26th March, 1973 were examined for the virus isolation. Though the virus isolation from hibernated female vector mosquitoes was made successfully in this period, no virus could be isolated from these ticks and lice.

DISCUSSION AND SUMMARY

When the virus isolation from the vector mosquitoes in Nagasaki area was made during two weeks in July 1973, the four strains of JE virus persisted with hibernated female mosquitoes of *Culex tritaeniorhynchus* caught in Koniya village located in the southern part of Amami island were detected in February in the interepidemic season of 1973. This findings have been surprising and suggestive for the consideration about overwintering of the virus in certain favourable conditions in the southern part of Japan. Furthermore, the close relation of the sequential infection with JE virus between mosquitoes and pigs was

observed through the year from 1973 to 1975 at the survey area in Amami island as seen in Fig. 5 and Fig. 6.

In 1974 and 1975, however, the virus isolation from vector mosquitoes was not made until the early part of July. These evidences indicated the interruption of the virus in the cycle of vector mosquitoes and pigs in winter, and also suggested that the virus might be carried again into the survey area during the epidemic season.

To attempt the collection of vector mosquitoes flying over the sea, the light traps were set up on the ship sailing between the southeast port in Kagoshima (Kyushu island) and the Amami island. The collection of mosquitoes was started on the sea at the positions of 50 km far each port. In the midnight on 24th to the very early morning on 25th July in 1973, 6 female mosquitoes of *Culex tritaeniorhynchus* mixed with 4 females and 19 males of *Culex pipiens fatigans* and 1 female of *Amopheles sinensis* happened to be captured. This finding suggested the transportation of the vector mosquitoes with the wind over the sea. In fact, it was reported by Asahina (1970) that the vector mosquitoes were captured in the East China Sea and the Pacific Ocean by weather-ships with the cooperation of the Maritime Meteorology Section, Maritime Division of Japan from 1968 to 1970. On the other hand, it was usually observed that migrating butterflies are transported over the Ocean and reach Kagoshima area, southern district of Kyushu island, in the late spring or the early summer every year. On the early part of July 1975, it was found the evidence that these migrating butterflies were ascertained by the identification of the emerged adults by Fukuda (1975).

On the stand point of these background of the transportation of the vector mosquitoes with wind blowing strongly into the southern part of the main island of Japan, the experimental dispersion of *Culex tritaeniorhynchus* was carried out in 1975.

An attempt to disperse about 100,000 mosquitoes of *Culex tritaeniorhynchus* labeled with fluorescent dyes was carried out from the north part of Amami island towards the southern part area of Kyushu island "it was actually towards Kagoshima area" on 25th July, 1975. The weather was selected the meteorological condition of the evening for the time of dispersion. For the detection of the labeled mosquitoes, the light traps were set up to collect mosquitoes in pigsheds and cowsheds at four islands "Takarashima-, Nakanoshima-, Yakushima-, and Tanegashima-islands" located between the south part of Kagashima and Amami island, furthermore, the four areas of the south part of Kagoshima. It was, however, unsuccessful with hindering by the occasionally happened typhoon.

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日本における日本脳炎ウイルスの生態学 Ⅲ. 1973年から1975年までの奄美大島における調査 : 林 薫, 三舟求真, 七条明久, 鈴木 博, 松尾幸子, 牧野 芳大, 明石光伸. (長崎大学熱帯医学研究所ウイルス学部門) 和田義人, 小田 力, 茂木幹義, 森 章夫. (長崎大学医学部医動物学教室)

1973年2月3日から18日の間, 新生成虫が検出されない時期に野外で捕集した冬期のコガタアカイエカ 1083個体, 8プールから4株のウイルスを分離し, 日本脳炎(日脳)ウイルスと同定された. この事実は, 越年蚊体内でウイルスが持ち越されたものと考えられる. そして1973年には年間を通じて, 蚊一豚の感染環が証明され, 奄美大島, 瀬戸内地域における日脳ウイルスの特異な撒布状況が観察された. この所見は我国で初めてのことである. しかしながら, 1974年には, コガタアカイエカから7月上旬にはじめてウイルスが分離されると共に, これと平行して豚の新感染も同時に証明された. この事は蚊一豚の感染環, 特に蚊によるウイルスの越年が中絶したことを意味すると共に, 奄美大島の調査地域へのウイルスの持込みがあったに違いないことを物語るものであろう. 換言すれば, 奄美大島の調査地域では環境条件さえよければウイルスの土着が可能であるが, 条件が悪いと蚊によるウイルスの越年は中絶し, 流行期に再びウイルスの持込みが行われるであろうことを推定してよいと思われる. 1973年7月24日夜半から25日未明にかけて奄美大島名瀬港及び鹿児島港の中間の海上で, 船のマスト上にとりつけられたライトトラップ採集でコガタアカイエカ 数個体を捕集した. この事実

はコガタアカイエカが洋上を移動していることを意味しているものと考えられる。1975年7月下旬、奄美大島から鹿児島（九州南域）に向け、標色コガタアカイエカの分散実験を試みたが、偶然に実験地域を通過した台風2号で阻止され不成功に終わった。しかし、分散実験日の約10日前にフィリッピンからの迷蝶が鹿児島南端に到達していることから気流によるコガタアカイエカの移動は決して否定出来ない。

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