



Title	1977年,沖縄本島における越冬コガタアカイエカからの日本脳炎ウイルス分離の試みについて
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Ecology of Japanese Encephalitis Virus in Okinawa, Japan

3. Supplementary note on the attempt of the isolation of Japanese encephalitis virus from hibernated female mosquitoes of *Culex tritaeniorhynchus* in Okinawa island in 1977

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Abstract: In the previous paper (Ura, 1977), it was pointed out that Japanese encephalitis (JE) viruses associated with hibernated female mosquitoes of *Culex tritaeniorhynchus* were isolated in February, 1976, at the northern district of the main island of Okinawa. Following this findings, JE virus dissemination at the survey area was demonstrated through the year in 1976. The serial survey was carried out at the same survey station, particularly, in the interepidemic season, from January 20 through March 1 in 1977. The virus isolation from 42 pools of 1,935 hibernated vector mosquitoes was unsuccessful. However, on May 12/13 and 20/21, 1977, JE virus was isolated from mosquitoes of *Culex tritaeniorhynchus* caught in the field and pig-sheds. These findings suggested that the persistence of the virus in vector mosquitoes was interrupted in winter season in 1977, and the virus might be carried again in the survey area in the epidemic season.

Serial surveys on the ecology of Japanese encephalitis (JE) virus, particularly, on the overwintering problems of the virus have been carefully carried out at Ogimi village located at the northern district of Okinawa island in 1977. The virus isolation from 42 pools of 1,935 hibernated females of vector mosquitoes was examined before the appearance of newly emerged vector mosquitoes usually identified in the middle part of March, every year. However, it was unsuccessful. On the other hand, the virus isolation from vector mosquitoes was made on May 12/13 and 20/21, in 1977. The results of these investigations will be described and discussed about the ecology of JE virus in Okinawa island in this paper.

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MATERIALS AND METHODS

Mosquito collection

(1) *Dry ice method*: Mosquito net (160 cm high, 140 cm wide, 185 cm long in size) supported on poles was set up in the field and dry ice was generated by throwing into some water in the mosquito net as the method described by Omori *et al.* (1965). Usually, it was generated 30 minutes before sunset and then continued about 2-3 hours.

(2) *Light trap method*: Two light traps (FHK super type s) were employed through the survey periods and set up in pig-pens at Ogimi village located at the northern district of Okinawa island. The traps were usually operated from the dusk to the next early morning. The mosquitoes collected by light traps were kept alive for ten days by feeding on 2% sugar solution for the digestion of a blood contained the antibodies which might interfere with the virus isolation from the mosquitoes.

Virus isolation

The female mosquitoes of *Culex tritaeniorhynchus* were pooled according to the collection methods and emulsified by the slightly modified method described by Hayashi *et al.* (1964). The emulsion of vector mosquitoes was made in the phosphate-buffered saline containing NaCl 8.0g, KCl 0.2g, Na₂HPO₄ 1.15g, KH₂PO₄ 0.2g per liter, at pH 7.4, 300 units of penicillin, and 300 mg of streptomycin per ml, and 0.75% bovine plasma albumin.

The suspensions holding in a refrigerator (4°C) were centrifuged at 10,000 rpm for 30 minutes in a refrigerated centrifuge with a rotor of the radius of 97 mm. Two or 3 day suckling mice were inoculated intracerebrally with 0.02 ml of the supernatant solution and the mice were observed for 14 days after inoculation.

Temperature and humidity

The weather conditions investigated in 1976 and 1977 were kindly presented by the Weather Bureau in Naha and the meteorological station located in Nago city at the northern part of Okinawa island.

RESULTS

In the main island of Okinawa, the newly emerged vector mosquitoes have been usually detected in the middle part of March (Ura, 1976). Accordingly, the vector mosquitoes in winter season were also carefully collected through January to February in 1977. The attempt for the virus isolation from 17 pools of 795 vector mosquitoes caught by the dry ice method and 25 pools of 1,140 vector mosquitoes caught by light traps both at the Ogimi village was made. However, it was not successful as seen in Table 1. In total, 5,558 mosquitoes by species were collected at the survey station as shown in Table 2.

On the other hand, on May 12/13 and 20/21, 1977, 6 pools of 264 vector mosquitoes and 4 pools of 172 vector mosquitoes were examined for the virus isolation, and 6 and 3 isolates from them were obtained respectively (Table 3).

These findings should be referred to the results which the virus could be isolated from

Table 1. Virus isolation from *Culex tritaeniorhynchus* mosquitoes caught by dry ice method in the field (A) and by light traps at pig-sheds (B) from 20th January to 1st March in 1977

A) Dry ice method			
Date	Number of mosquitoes	Number of pools	Number of isolates
Jan. 20	1	} 3	0
Feb. 6	1		
12	135		
13	632	13	0
28	26	1	0
Total	795	17	0

B) Light trap method			
Date	Number of mosquitoes	Number of pools	Number of isolates
Jan. 20/21	6	1	0
Feb. 6/7	28	1	0
12/13	359	8	0
13/14	547	11	0
28/Mar. 1	200	4	0
Total	1,140	25	0

Table 2. Number of mosquitoes by species collected by a light trap at Ogimi village

Species	6 - 7	12 - 13	13 - 14	28 - Mar. 1	Total
<i>Culex fatigans</i>	1	195	72	20	288
<i>Culex tritaeniorhynchus</i>	18	2,683	2,253	158	5,112
<i>Aedes vexans</i>	3	12	45	—	60
<i>Anopheles sinensis</i>	11	35	17	31	94
<i>Mansonia ochracea</i>	1	—	—	—	1
<i>Armigeres subalbatus</i>	—	—	3	—	3
	34	2,925	2,390	209	5,558

Remarks : Mosquitoes were collected through the overnight (from the evening to the early morning) at 6th, 12th, 13th and 28th February, 1977.

Table 3. Virus isolation from vector mosquitoes caught in Okinawa island in the epidemic season in 1977

Date	Number of mosquitoes	Number of pools	Number of isolates
May 12/13	264	6	6
∕ 20/21	172	4	3

The vector mosquitoes were caught by light traps at the pig-pens at Ogimi village.

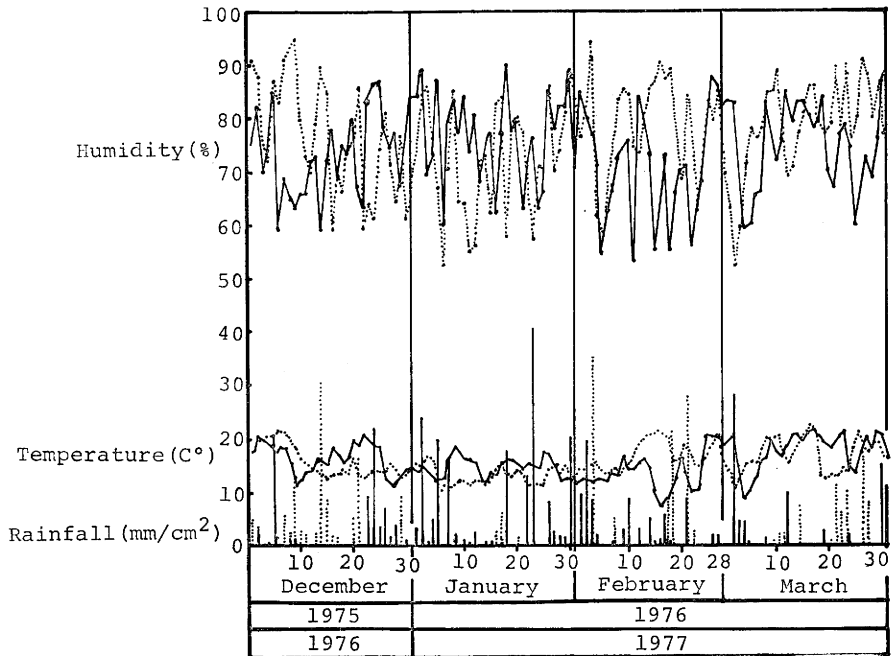


Fig. 1 Daily humidity, temperature and rainfall in the northern part of Okinawa island from December 1976 to March 1977. The dotted and solid lines show the daily records of humidity, temperature and rainfall from December, 1975 to March, 1976 and from December, 1976 to March, 1977, respectively.

the hibernated female mosquitoes of *Culex tritaeniorhynchus* in the interepidemic season in 1976. First of all, in view of the natural circumstances which is considered to influence on the surviving of hibernating vector mosquitoes in winter season, the weather conditions must be examined. According to the reports of weather conditions in winter season in 1976 and 1977 presented by the meteorological station at Nago city located in the northern part of the island, the daily temperatures in January indicated without great alteration between 1976 and 1977 (Fig. 1). However, the temperature in February 1977 indicated the remarkable changing than in 1976 as shown in Fig. 2. These intensely alteration of lower temperatures at the survey area in winter season in 1977 might interrupt the persistence of JE virus in vector mosquitoes.

DISCUSSION

It was surprising and suggestive evidence that JE virus was demonstrated to persist with hibernated female mosquitoes of *Culex tritaeniorhynchus* caught in Amami island in February in 1973 (Hayashi, *et al.* 1975). However, in 1974 and 1975, the virus isolation from vector mosquitoes was made on the middle part of July in the epidemic season. These finding indicated the interruption of the virus in the vector mosquitoes in winter season at the survey station in Amami island.

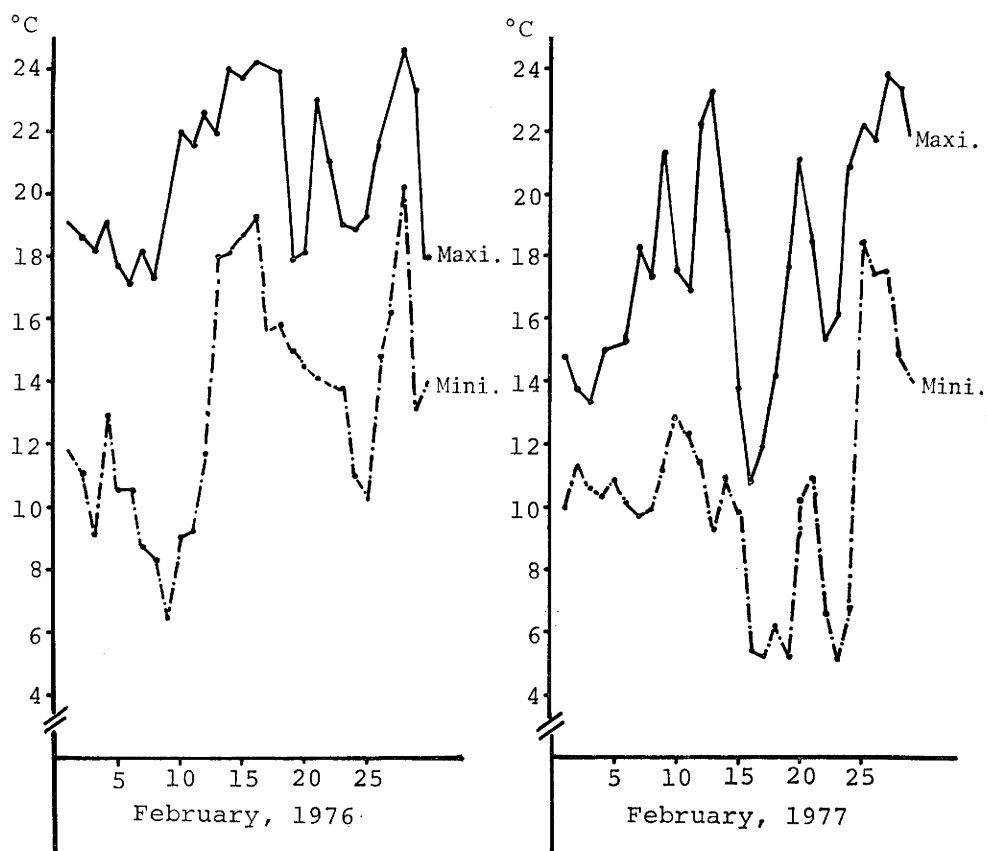


Fig. 2 Daily minimum and maximum temperature at the Nago meteorological station in the northern part of Okinawa island.

In the previous paper (Ura, 1976), it was reported as the noticeable fact that JE virus was isolated from overwintered female vector mosquitoes caught in February, 1976, at Ogimi village located in the northern district of the main island of Okinawa. Serial investigations at the same survey area was also carefully made in the interepidemic season in 1977, and the virus isolation from hibernated vector mosquitoes caught in January and February in 1977 was unsuccessful. However, the virus was isolated on the middle part of May, 1977, in the epidemic season. It was noted that these evidences demonstrated in the northern district of Okinawa island was similar to the findings investigated in Amami island from 1973 to 1975. It was suggested that JE virus might also persist in vector mosquitoes in winter season in certain favourable conditions in Okinawa island. It was, however, considered that such a persistence of the virus in hibernated vector mosquitoes might be interrupted by certain severe conditions in winter season and the virus might be carried again in the survey area during the epidemic season.

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1977年、沖縄本島における越冬コガタアカイエカからの日本脳炎ウイルス分離の試みについて
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1976年2月、沖縄本島北部で捕集した越冬コガタアカイエカから日本脳炎ウイルスが分離され、同年は引続いて年間を通じ蚊及び豚血清からウイルスが分離された。このことは前年の1975年冬期から1976年にかけて日本脳炎ウイルスが沖縄本島の調査地点に土着していたことを示す事実であることについて前報で詳述した。そして、このような所見は奄美大島について日本で二番目の知見である。また1977年も引続いて調査し、特に冬期の蚊1,905個体42プールについてウイルス分離を試みたが今回は陰性に終わった。そして例年と同じく5月中旬以降になって野外蚊から日本脳炎ウイルスが分離された。この事実は、1977年では冬期に日本脳炎ウイルスの生存環が中絶し、流行期に再び調査地にウイルスの持込みがあったことを物語っているものと推定することが出来た。

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