

## Ecology of Japanese Encephalitis Virus in Okinawa, Japan

### II. Regional particularity of the virus dissemination in Okinawa island from 1966 to 1976

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**Abstract:** In the previous paper, it was indicated that the mosquito and pig infection in Okinawa island has usually been demonstrated from early April or May and vanished in late September or middle October since 1966. It was also pointed out that such a long period of JE virus dissemination in nature was never observed in Nagasaki area as well as in the main island of Japan. In this paper, the local peculiarity of JE virus dissemination by area in Okinawa island since 1966 will be described. The virus isolation from pig-sera and vector mosquitoes and the detection of 2-mercaptoethanol (2ME) sensitive antibody in pig-sera in every epidemic season were usually made earlier in the northern area than in the middle and southern areas of the island. In 1976, JE virus was isolated from the overwintered female vector mosquitoes caught in February as indicated in the previous paper. This noticeable evidence was demonstrated in the northern area of the island.

It was reported that the earliest isolation of JE virus from mosquitoes of *Culex tritaeniorhynchus* in Japan was made on 19th and 30th May in 1965 in Nagasaki area (Takahashi *et al.* 1968; Hayashi *et al.* 1965) and the latest one was demonstrated on 5th or 6th September in 1963 or 1965 in Kanto or Nagasaki area respectively (Oya *et al.* 1963; Shichijo *et al.* 1968). However, in the recent investigation on JE virus dissemination, it was noted that the mosquito infection in Nagasaki area and also in the whole of Japan was demonstrated from the middle or the late part of July to the early or middle part of August, especially since 1973 (Fukumi *et al.* 1975; Wada *et al.* 1975; Hayashi *et al.* 1976). On the other hand, in Okinawa island, the isolation of JE virus from pig-sera and vector mosquitoes was usually made from April or May to October or November (Ura, 1976). In this paper,

such a local peculiarity of the virus dissemination in Okinawa island was observed for the northern, middle and southern parts of the island and the localized situation of the ecology of JE virus will be discussed.

#### MATERIALS AND METHODS

As shown in Fig. 1, Okinawa island was bounded into three districts for the geographical convenience; namely, northern area north of Nago district containing a city, a town and 7 villages, middle area including from Ginowan and Nakagusuku to Onna and Ginoza districts containing 4 cities, a town and 9 villages, southern area south of Naha district containing 3 city, a town and 14 villages. Pig-sera were collected at three slaughter houses located in the northern area (Nago), middle area (Gushikawa), and southern area (Madambashi on Ozato). Pig-sera were also identified for their breeding farms as shown in Fig. 1. Vector mosquitoes were caught by light traps at the pigsheds located in each area and also dry ice method was used in the field from January to March since 1973. The methods for the examination of hemagglutination inhibition (HI) and 2-mercaptoethanol (2ME) sensitive



Fig. 1 Map of Okinawa island and survey places.

antibodies in pig-sera and for the virus isolation from pig-sera and vector mosquitoes were described in the previous paper.

## RESULTS

### **Virus dissemination by local area from 1966 to 1968**

The examination of pig-sera for HI and 2ME sensitive antibodies was fixed from 1966 to 1968, because there were difficulties to isolate the virus from pig-sera and vector mosquitoes due to the insufficient equipment during these 3 years. So far as the detection of HI and 2ME sensitive antibodies was concerned, it was indicated that JE virus might have been disseminated from the middle or the late part of April in these 3 years to the late part of September in 1966 (Fig. 2, 3a, 3b). 2ME sensitive antibodies were detected in pig-sera collected from January to March and after October in these 3 years especially at the northern area. These events suggested the pig infection with the virus in the interepidemic season. However, the identification of 2ME sensitive antibody in pig-sera must be made carefully by using the methods of 2ME-iodoacetoamide treatment and analytical centrifugation. In addition, it will also be necessary to isolate the virus from pig-sera and vector mosquitoes in the interepidemic season for the improvement of the overwintering problems of JE virus in the island.

### **Virus dissemination by local area from 1969 to 1972**

Even though the date of the virus isolation from pig-sera and vector mosquitoes was different in these 4 years (April 16, 1969; June 2, 1970; May 11, 1972), it was found that the isolation of JE virus was usually made earlier in the northern area than in the middle and southern areas of the island. It was also recognized that the detection of HI antibody particularly 2ME sensitive antibody in slaughtered pig-sera was made closely in parallel with the mosquito infection and with the events of the virus isolation from pig-sera. So far as the detection of 2ME sensitive antibodies in pig-sera was concerned, it was indicated that the possibility of vector mosquito and pig infection in the interepidemic season in these 4 years would be presumed in the southern area especially in the northern area (Table 1, Fig. 4a, 4b; 5a, 5b; 6a, 6b; 7a, 7b).

### **Virus dissemination by local area from 1973 to 1976**

In 1973, in the northern and middle areas, the virus isolation from vector mosquitoes and pig-sera was made on 7th and 19th July respectively. In contrast, the 2ME sensitive antibody in pig-sera was found in the late part of May in the northern area. However, the 2ME sensitive antibody in the sera of pigs bred and slaughtered in the southern area was detected in January and February in 1973. It might be suggested that JE virus was disseminated in the southern localized area in this year (Table 2, Fig. 8a, 8b). In 1974 and 1975, the mosquito and pig infection was observed from the middle part of May to the late part of October and the early part of November in the northern area. In the middle and southern areas, the virus isolation from pig-sera and vector mosquitoes and also the

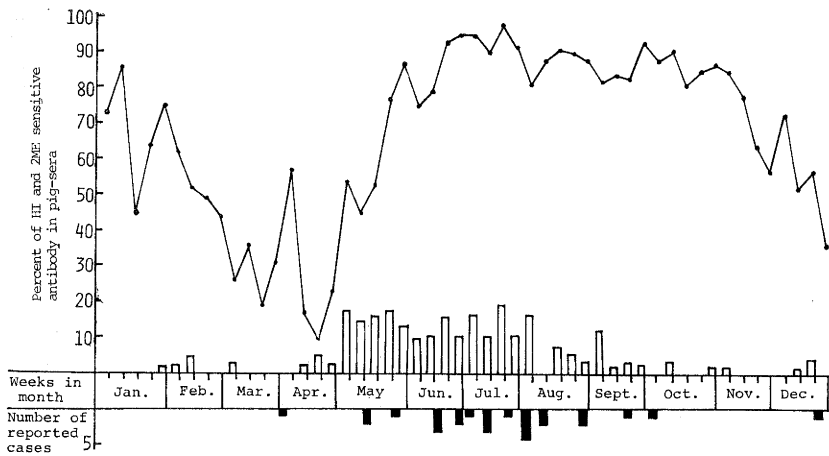


Fig. 2. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in the main island of Okinawa in 1966.

Remarks : The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The black sticks mean the number of human Japanese encephalitis cases.

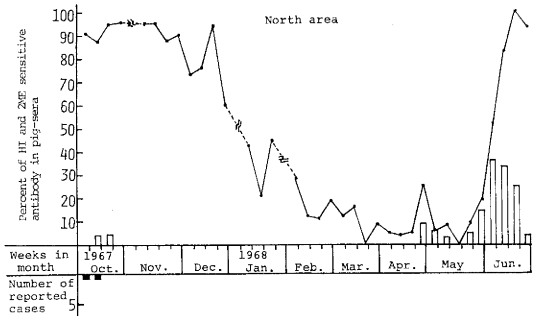
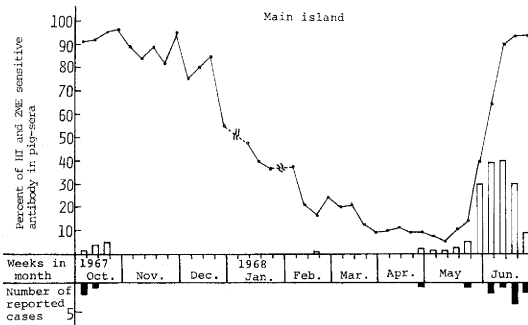


Fig. 3a. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in Okinawa island from 1968.

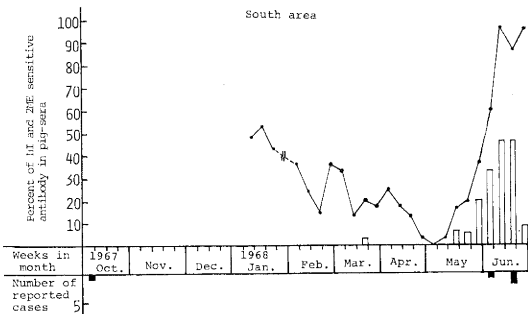
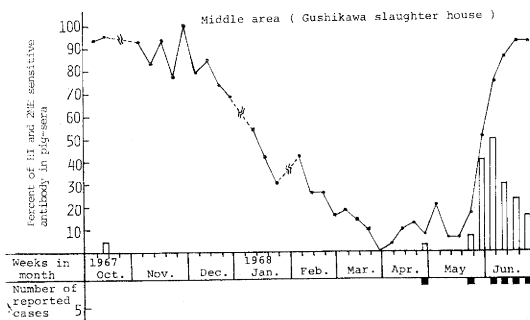


Fig. 3b.

Remarks : The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The black sticks mean the number of human Japanese encephalitis cases.

detection of 2ME sensitive antibody in pig-sera were made from June to September (Table 2, Fig. 9a, 9b; 10a, 10b). It was indicated that the virus dissemination in the northern area might have been earlier and longer than that in the middle and southern areas in these 2 years. In 1976, 2 strains of JE virus could be isolated from overwintering female mosquitoes of *Culex tritaeniorhynchus* caught in the northern area on 13th/15th February as described in the previous paper. It was suggested that JE virus might have overwintered persisting in the hibernated female vector mosquitoes and there might have been preserved some particular situation in the northern area of the island. It was also noted that the virus isolations from vector mosquitoes in the middle and southern areas were made on the late part of May and July in this year (Fig. 11a, 11b).

Table 1 Detection of 2ME sensitive antibody and virus isolation from pig-sera by month in the main island of Okinawa from 1969 to 1972

Year	1969			1970			1971			1972		
	number of sera tested	number of 2ME sensitive sera	virus isolation from pig-sera and mosquitoes	number of sera tested	number of 2ME sensitive sera	virus isolation from pig-sera and mosquitoes	number of sera tested	number of 2ME sensitive sera	virus isolation from pig-sera and mosquitoes	number of sera tested	number of 2ME sensitive sera	virus isolation from pig-sera and mosquitoes
January	117	2(S)	0	120	0	0	90	0	0	86	5(S)	0
February	105	1(S)	0	120	0	0	120	1(N)	0	90	0	0
March	103	1(N)	0	145	0	0	150	0	0	150	1(N)	0
April	117	3 <sup>(N)</sup> <sub>(S)</sub>	1(N)	120	0	0	120	0	0	118	1(N)	0
May	97	14 <sup>(N)</sup> <sub>(S)</sub>	2(S)	119	2(S)	0	119	3(S)	5 <sup>(N)</sup> <sub>(S)</sub>	146	2(N)	0
June	124	24 <sup>(N)</sup> <sub>(M)</sub> (S)	1(S)	149	17 <sup>(N)</sup> <sub>(S)</sub>	8 <sup>(N)</sup> <sub>(S)</sub>	150	28 <sup>(N)</sup> <sub>(M)</sub> (S)	32 <sup>(N)</sup> <sub>(M)</sub> (S)	103	1(S)	0
July	n.d.			60	0	0	60	14 <sup>(N)</sup> <sub>(S)</sub>	0	86	10 <sup>(N)</sup> <sub>(S)</sub>	2 <sup>(N)</sup> <sub>(S)</sub>
August	∕			60	0	0	30	1(S)	1(S)	150	35 <sup>(N)</sup> <sub>(S)</sub>	4 <sup>(M)</sup> <sub>(S)</sub>
September	∕			59	0	0	30	1(S)	1(N)	119	28 <sup>(N)</sup> <sub>(S)</sub>	5 <sup>(N)</sup> <sub>(S)</sub>
October	∕			59	0	0	30	1(S)	0	90	19 <sup>(N)</sup> <sub>(S)</sub>	4 <sup>(N)</sup> <sub>(S)</sub>
November	∕			30	0	0	29	0	0	57	0	
December	∕			60	0	0	30	0	0	59	0	
Total	663	45	4	1,101	19	8	958	49	39	1,254	100	15

Remarks : (N).....North area (M).....Middle area (S).....South area

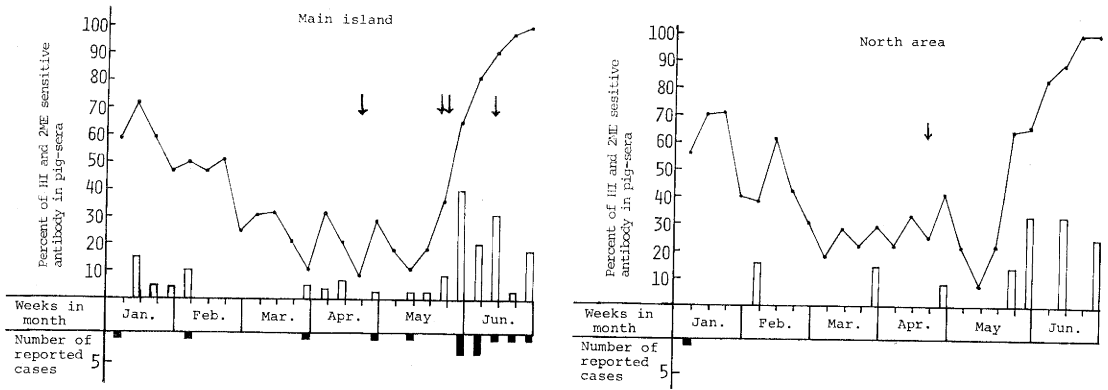


Fig. 4a. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in Okinawa island in 1969.

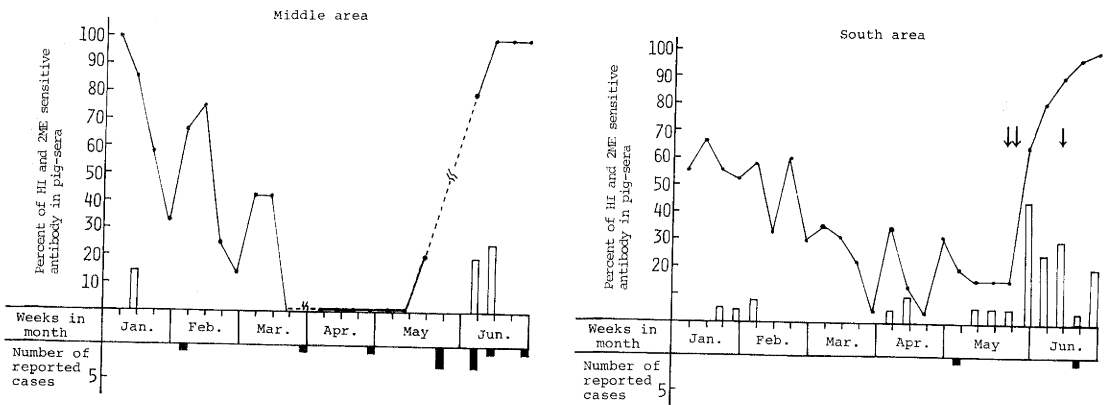


Fig. 4b.

Remarks: The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The arrows mean the virus isolation from slaughtered swine sera and mosquitoes of *Culex tritaeniorhynchus*. The black sticks mean the number of human Japanese encephalitis cases.

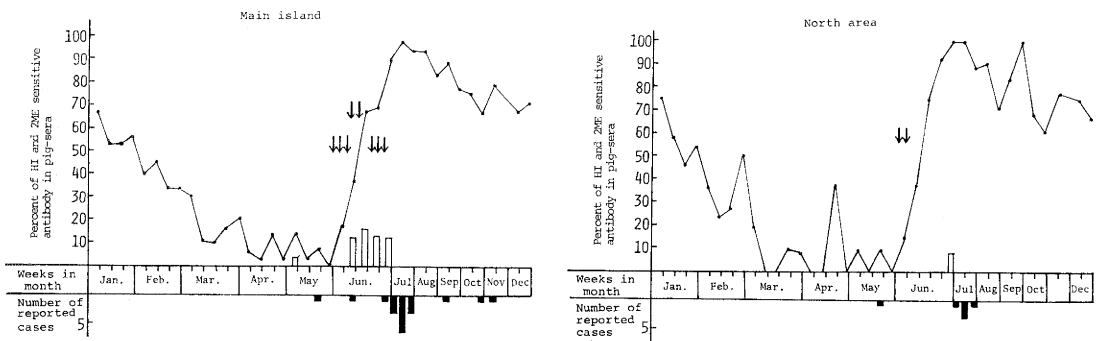


Fig. 5a. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in Okinawa island in 1970.

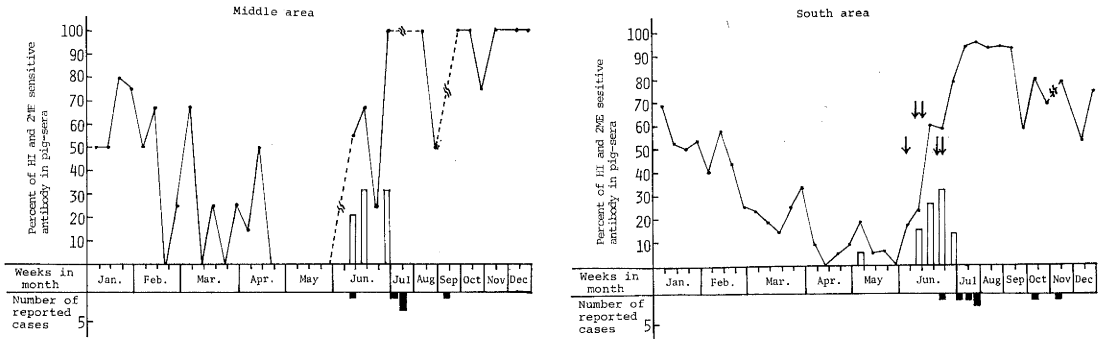


Fig. 5b.

Remarks: The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The arrows mean the virus isolation from slaughtered swine sera and mosquitoes of *Culex tritaeniorhynchus*. The black sticks mean the number of human Japanese encephalitis cases.

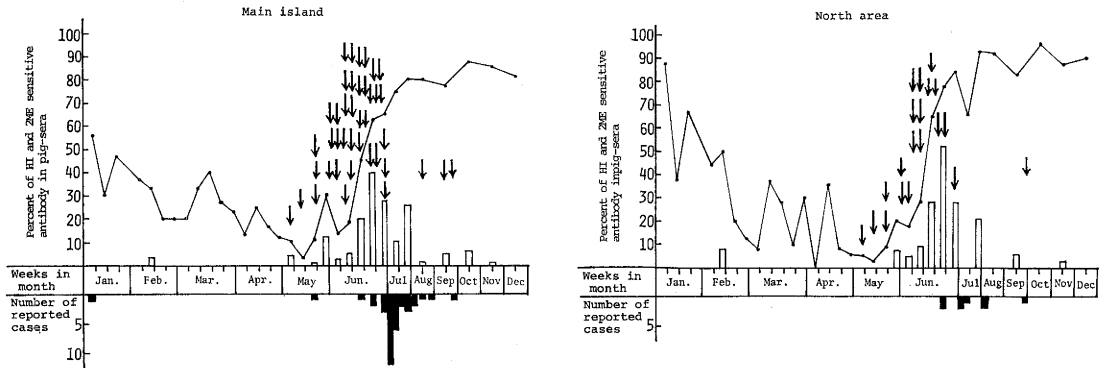


Fig. 6a. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in Okinawa island in 1971.

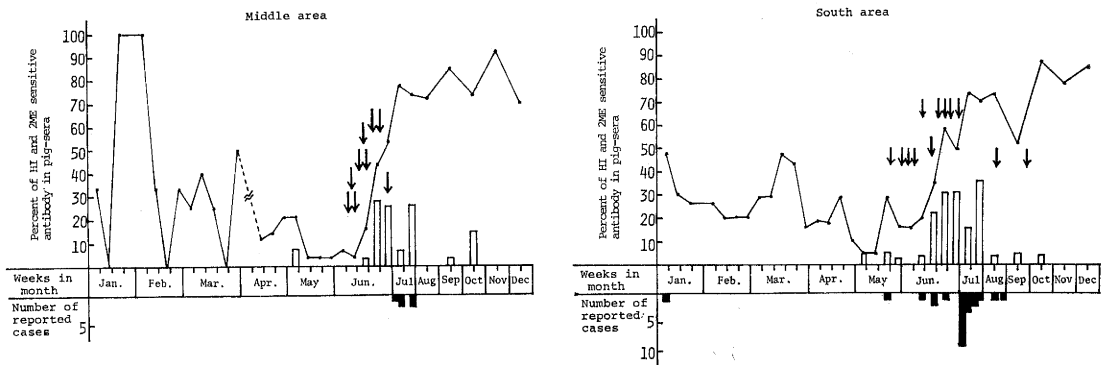


Fig. 6b.

Remarks: The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The arrows mean the virus isolation from slaughtered swine sera and mosquitoes of *Culex tritaeniorhynchus*. The black sticks mean the number of human Japanese encephalitis cases.

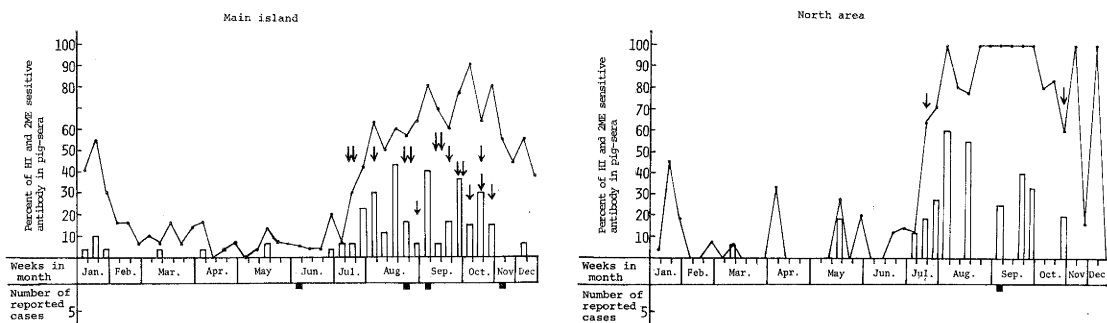


Fig. 7a. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in Okinawa island in 1972.

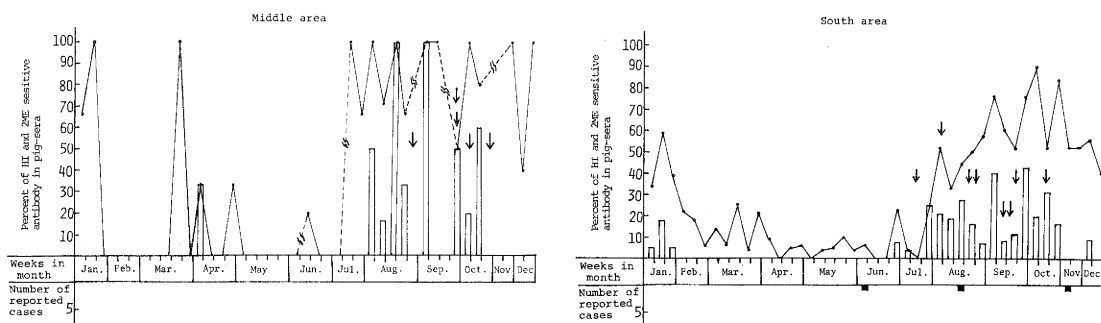


Fig. 7b.

Remarks: The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The arrows mean the virus isolation from slaughtered swine sere and mosquitoes of *Culex tritaeniorhynchus*. The black sticks mean the number of human Japanese encephalitis cases.

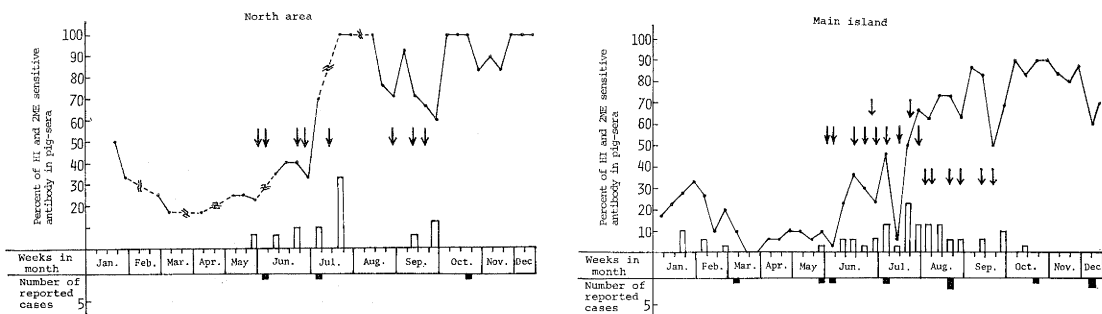


Fig. 8a. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in Okinawa island in 1973.



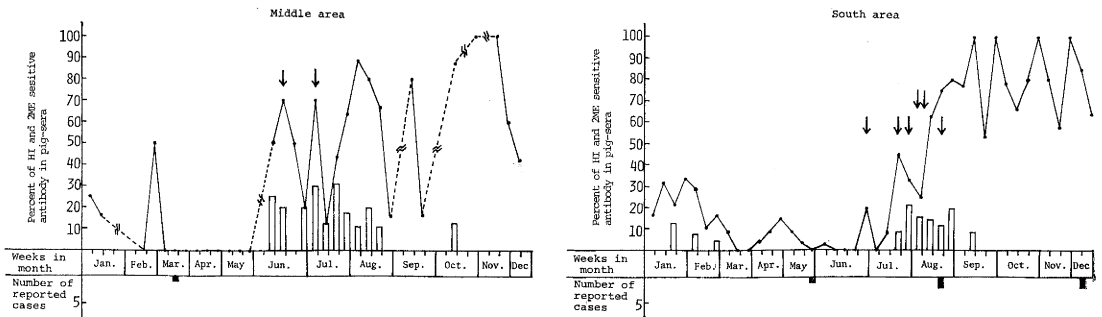


Fig. 8b.

Remarks: The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The arrows mean the virus isolation from slaughtered swine sera and mosquitoes of *Culex tritaeniorhynchus*. The black sticks mean the number of human Japanese encephalitis cases.

Table 2 Detection of 2ME sensitive antibody and virus isolation from pig-sera by month in the main island of Okinawa from 1973 to 1976

Year	1973			1974			1975			1976			
	Month	number of sera tested	number of 2ME sensitive sera	virus isolation from pig-sera and mosquitoes	number of sera tested	number of 2ME sensitive sera	virus isolation from pig-sera and mosquitoes	number of sera tested	number of 2ME sensitive sera	virus isolation from pig-sera and mosquitoes	number of sera tested	number of 2ME sensitive sera	virus isolation from pig-sera and mosquitoes
January		116	3(S)	0	90	0	0	90	0	0	0	0	0
February		90	3(S)	0	120	0	0	120	0	0	0	0	2(N)
March		90	0	0	90	0	0	120	0	0	0	0	0
April		90	0	0	120	0	0	115	0	0	30	0	2(N)
May		90	1(N)	0	150	5(N)	1(N)	155	0	2(N)	90	4 <sup>(N)</sup> <sub>(M)</sub>	6(N)
June		149	7 <sup>(N)</sup> <sub>(M)</sub> (S)	5 <sup>(N)</sup> <sub>(M)</sub>	120	10 <sup>(N)</sup> <sub>(M)</sub>	4(N)	120	8 <sup>(N)</sup> <sub>(M)</sub>	3(N)	90	10 <sup>(N)</sup> <sub>(M)</sub>	5(N)
July		120	16 <sup>(M)</sup> <sub>(S)</sub>	4 <sup>(N)</sup> <sub>(M)</sub> (S)	120	15 <sup>(N)</sup> <sub>(S)</sub>	6(S)	120	10 <sup>(N)</sup> <sub>(M)</sub> (S)	4 <sup>(N)</sup> <sub>(S)</sub>	60	5(N)	4(N)
August		120	12 <sup>(N)</sup> <sub>(S)</sub>	4 <sup>(N)</sup> <sub>(S)</sub>	120	20 <sup>(N)</sup> <sub>(S)</sub>	1(S)	150	16 <sup>(N)</sup> <sub>(S)</sub>	3(S)	0	0	0
September		119	5 <sup>(N)</sup> <sub>(M)</sub> (S)	2(N)	90	3(S)	0	120	4 <sup>(N)</sup> <sub>(S)</sub>	0	30	1(M)	2 <sup>(N)</sup> <sub>(S)</sub>
October		120	1(N)	0	120	2 <sup>(N)</sup> <sub>(S)</sub>	0	150	1(N)	1(N)	60	3 <sup>(M)</sup> <sub>(S)</sub>	0
November		90	0	0	90	0	0	90	1(N)	0	30	1(M)	0
December		60	0	0	60	0	0	60	0	0	n.d.	n.d.	n.d.
Total		1,254	42	12	1,290	45	9	1,410	40	12	390	24	21

Remarks : See Table 1.

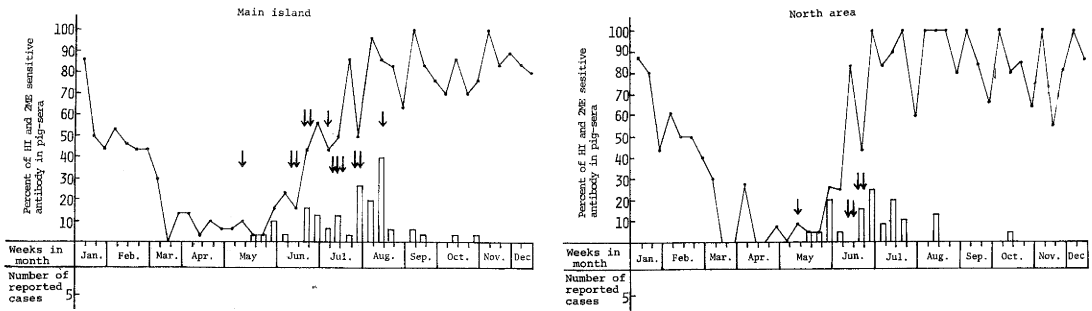


Fig. 9a. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in Okinawa island in 1974.

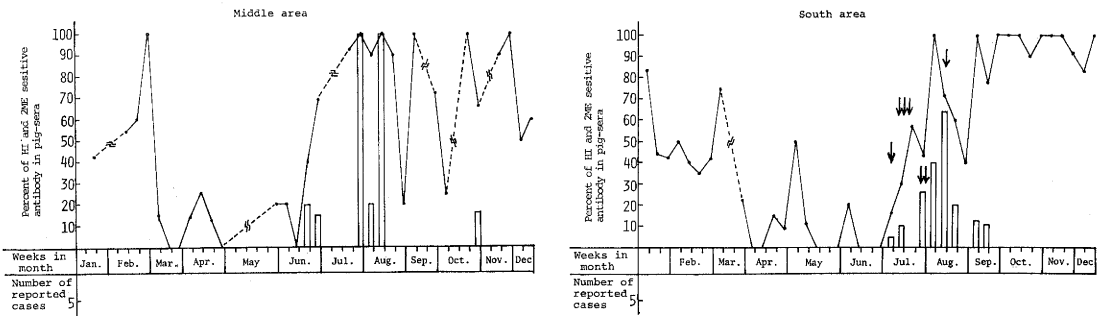


Fig. 9b.

Remarks: The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The arrows mean the virus isolation from slaughtered swine sera and mosquitoes of *Culex tritaeniorhynchus*.

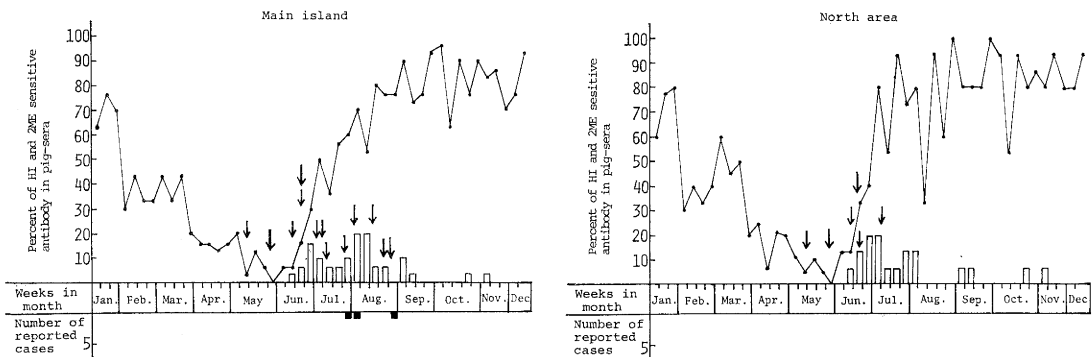


Fig. 10a. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in Okinawa island in 1975.

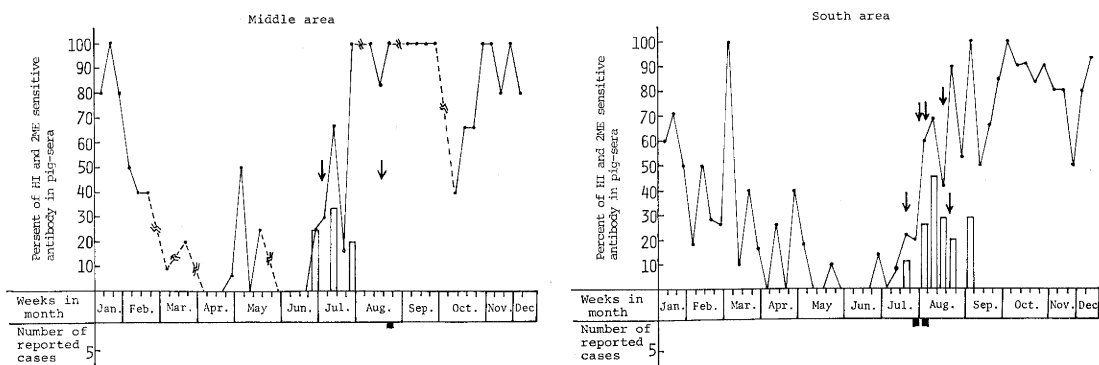


Fig. 10b.

Remarks: The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The arrows mean the virus isolation from slaughtered swine sera and mosquitoes of *Culex tritaeniorhynchus*. The black sticks mean the number or human Japanese encephalitis cases.

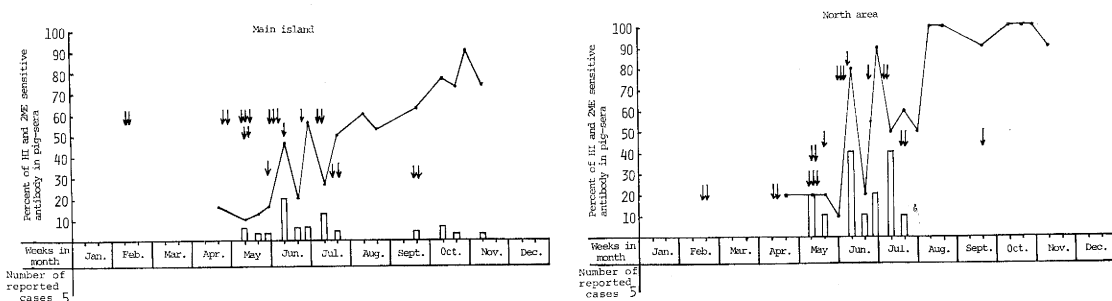


Fig. 11a. HI antibody and 2ME sensitive antibody in slaughtered swine sera collected in Okinawa island in 1976.

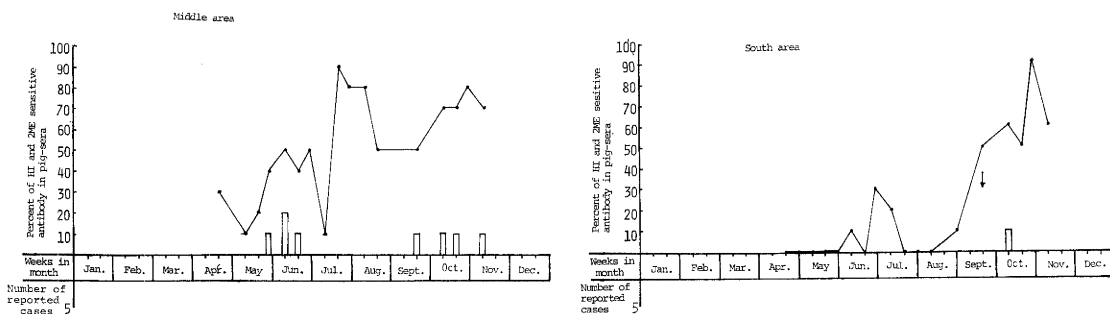


Fig. 11b.

Remarks: The dotted line means the rate of HI positive in swine sera and white stick means the rate of 2ME sensitive antibody possess in them. The arrows mean the virus isolation from slaughtered swine sera and mosquitoes of *Culex tritaeniorhynchus*.

## DISCUSSION

The variation in time and period of virus isolation from vector mosquitoes by place in the epidemic season was investigated in Nagasaki area in 1965 (Hayashi *et al.* 1965). It was also indicated that the pig infection informed by HI antibody rising in the pig-sera has usually extended from the southern part to the northern area of Japan (Hayashi, *et al.* 1975). However, there has been little knowledge about continuous and detailed investigations on the local particularity of JE virus dissemination in the limited places. It was demonstrated by the author in the previous paper that the time and period of JE virus dissemination in Okinawa island located in subtropical area was earlier and longer by about two or three months than in Nagasaki area and in the whole of Japan (Ura, 1976). In this paper, it is reported that the local peculiarity of JE virus dissemination in the northern, middle and southern areas of Okinawa island was investigated.

When the potential motion of JE virus in the interepidemic season was focused on the detection of the 2ME sensitive antibody in pig-sera, it might be presumed that the pigs happen to be infected with the virus at that time. On the standpoint of such a consideration, it was noticed that the 2ME sensitive antibody was detected in the pig-sera collected in the northern area and occasionally in the southern area of the island from January to March since 1966 (Fig. 2a, 3a, Table 1, 2). Following these events in the interepidemic season, the virus isolation from pig-sera and mosquitoes was always made earlier in the northern area than in the middle and southern areas in every epidemic season. Iha (1971) has reported that in the main island of Okinawa, the gonoactivity of *Culex tritaeniorhynchus* female mosquitoes seems little affected with shortening of day length in cold winter months and to continue feeding activity in warmer temperature during winter season whereas, the female mosquitoes of *Culex tritaeniorhynchus* in Nagasaki area definitely affected with shortening of day length in spite of the environmental temperature (Kawai, 1969). Furthermore, it was a noticeable finding that in the northern area, the virus isolation from overwintered female mosquitoes of *Culex tritaeniorhynchus* caught in the field in the northern area was made as presented in the previous paper (Ura, 1976). These events might have been related to the industrial development of the island. Accordingly, the rice-field area remarkably decreased in extent especially in the middle and southern areas of the island, and it may be considered that the source for the breeding of the vector mosquitoes diminished since 1973. In contrast, it was considered that certain favourable circumstances particularly for the overwintering of the virus in interepidemic season must have remained in the northern area of the island.

## 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.

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## 沖縄における日本脳炎ウイルスの生態の研究

## Ⅱ. 沖縄本土における日本脳炎ウイルスの撒布の地域特異性

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前報で沖縄本島では豚血清及びコガタアカイエカから日脳ウイルスが分離される期間は例年4月上旬か5月上旬から9月下旬あるいは10月中旬までであって、ウイルス撒布期間は著しく長期に亘ることを明かにした。このように流行期の始まりが早いことやウイルス汚染期間が長いことは長崎地方を含めて日本本土では決して認められない現象であることを指摘した。本報では、沖縄本島におけるウイルス撒布の状況を地域別に詳細に観察した所見を述べた。即ち、沖縄本島を南部、中部、北部地域に区分し、豚血清及び蚊から最初にウイルスが分離されるのは常に北部地域であって、中部及び南部地域はこれよりややおくることが判かった。豚血清中の2ME感受性抗体の検出状況やその保有率もウイルス分離の所見と平行していた。1976年、北部地域では2月13日/15日に野外で捕集した越冬コガタアカイエカから日脳ウイルスが分離され、北部地域では4月以後年間を通じて蚊及び豚血清からウイルスが分離されると共に豚血清中の2ME感受性抗体も平行して検出された。これに比べ中部及び南部地域のウイルス汚染は北部地域よりおくれ5月下旬から始まった。このように北部地域は1976年には年間、ウイルスの生存環が保持されたことを示していた。亜熱帯地帯に位置し、かつ比較的狭小な島である沖縄本島において日脳ウイルスの撒布の様相は例年定常的といえるほど明瞭な地域差を示すことを明かにした。

熱帯医学 第18巻 第4号 165-178頁, 1976年12月