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Ecology of Japanese Encephalitis Virus in Nagasaki Area, Okinawa and Amami Islands

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ABSTRACT : Comparative investigations on the ecology of Japanese encephalitis (JE) virus have been made in Nagasaki area, Okinawa and Amami islands. In Nagasaki area, the virus dissemination in nature had been observed in a long duration (35 to 100 days per year) from 1964 to 1968, although it has been shortened up to two weeks after 1969 until now. In the interepidemic season, the overwintering of the virus still remains as a difficult problem. In the main island of Okinawa, the virus has disseminated about two or three months earlier in every epidemic season than in Nagasaki area. In Amami island, a peculiar evidence was found that JE virus was isolated from hibernated female mosquitoes of *Culex tritaeniorhynchus* caught in winter, (February), 1973, and thereafter the virus was continuously transmitted between vector mosquitoes and swine in that year. However, it was interrupted in the interepidemic season in 1974 and the virus isolation was made in the middle patrt of July, 1974. It was concluded that the localized persistence of the virus might be preserved under a suitable condition. However, when it was interrupted in winter, the virus might be carried again from elsewhere to the survey area. These findings may support the carried-in theory on the problems of overwintering of JE virus in Japan.

It is a well-known fact that Japanese encephalitis (JE) virus is wide spread in Asia including India, South China, Korea and Mariana islands. The favorable situation for the transmission cycle and the interepidemic persistence of JE virus might varied by area among the temperate (main islands of Japan and Korea), subtropical (Amami and Okinawa islands in Japan) and tropical (South East Asia) zones. It is cosidered that, in the temperate zone, there may be unique circumstances for the transmission of JE virus, particularly for the overwintering of the virus. In contrast, in the tropical zone, a continuous transmission cycle of JE virus to susceptible hosts may possibly be expected.

Inspite of the fact that the virus dissemination in the field has been decreased and shortened in terms every year since 1969 and the human cases of JE have also been decreased remarkably in number in main islands of Japan, it is still recognized that the virus isolation from vector mosquitoes and the detection of 2 mercaptoethanol sensitive

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antibody in swine sera have usually been made every year since 1970.

On the other hand, the ecology of the overwintering of JE virus is still unknown. While experimental attempts to verify the mode of overwintering of JE virus have been successful, no evidences have been obtained in the field up to now.

Comparative studies on the ecology of JE virus have been carried out in Nagasaki area and the main island of Okinawa since 1969 and also in Amami island since 1973. It was demonstrated that, in epidemic season, the virus dissemination in nature in the main island of Okinawa has always started earlier by about two or three months than that in Nagasaki area since 1969 until now. It was also noted that JE virus was isolated from hibernated female mosquitoes of *Culex tritaeniorhynchus* caught in the field in Amami island located in the East China Sea in February, 1973. Although a continous transmission cycle between vector mosquitoes and swine was confirmed in this island in 1973, it was interrupted in winter in 1974. In this paper, the results of comparative investigations on the ecology of JE virus in Nagasaki area, main island of Okinawa and Amami island will be described and, on the basis of these results, the problems of the overwintering of JE virus in Japan will be discussed.

RESULTS

1. The course of infection of vector mosquitoes and swine with JE virus.

When the vector mosquito bite the susceptible animal having a condition of viremia, the virus antigen could be observed in a few cells of midgut of the vector mosquito on the third day, in the whole cells on the seventh to tenth day, and in nervous cells, fat body cells and salivary glands on the eleventh to fifteenth day. With reference to the progress of infection in vector mosquitoes with the virus, it is obvious that the virus isolation from the infected mosquitoes can be successful on and after the third day (Shichijo, et al. 1972). It was also demonstrated that a susceptible swine was infected experimentally by biting with only an infected hibernated female mosquito of *Culex tritaeniorhynchus* (Mifune, 1965). The viremia in the sera of the infected swine appeared from the second to sixth day after infection. Thereafter, the 2ME resistant antibody (IgG) following the production of 2ME sensitive antibody (IgM) in the infected swine sera was manifested (Fig. 1).

These evidence were necessary for the basis of the ecological investigations of JE virus in nature.

2. Results of investigations on the ecology of JE virus in Nagasaki area from 1964 to 1974.

(1) Places for the collection of mosquitoes and swine sera.

As shown in Fig. 2, five places were usually selected for the survey of the virus dissemination in Nagasaki area.

(2) Investigation on the mosquito infection in Nagasaki area from 1964 to 1974.

The virus dissemination in nature as indicated by the mosquito infection were observed

in a long duration each year from 1964 to 1968. After 1968, the periods of the virus isolation from mosquitoes caught in the fields was shortened up to two weeks (Table 1, Fig. 3, 4). It has been found that the hibernated female mosquitoes of *Culex tritaeniorhynchus* usually appeared from the last part of March to April, and after that, the newly emarged mosquitoes were increased in number up to July every year. In spite of the similar finding on the seasonal prevalence of vector mosquitoes every year since 1965, the period of virus isolation from them varied between before 1968 and after 1969. Subsequently, human cases of JE have also been decreased in number since 1969. There is little knowledge about these different conditions for the virus dissemination in nature in connection with the occurrence of JE cases.

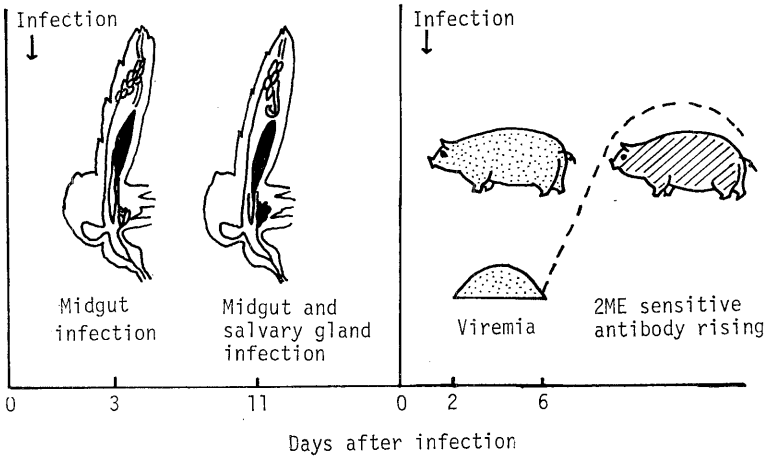


Fig. 1. JE virus infection of mosquitoes and swine.

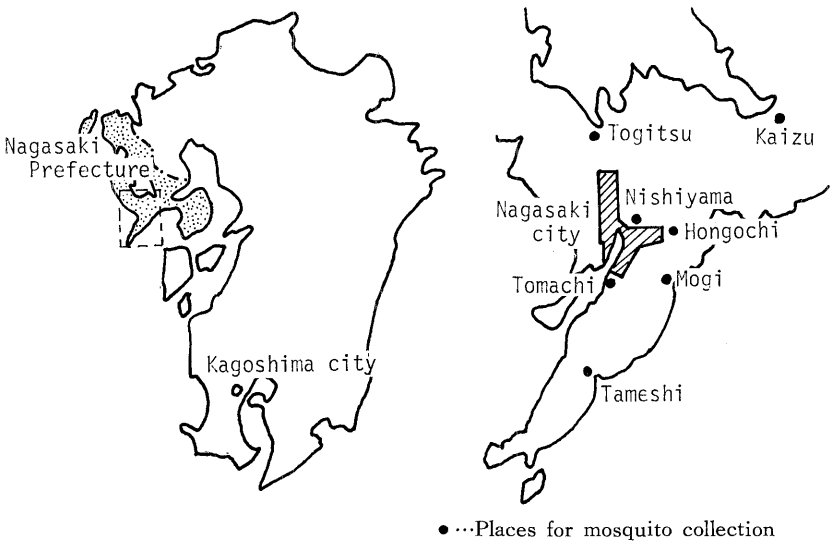


Fig. 2. Outline of Kyushu island and survey stations in Nagasaki and Kagoshima prefectures.

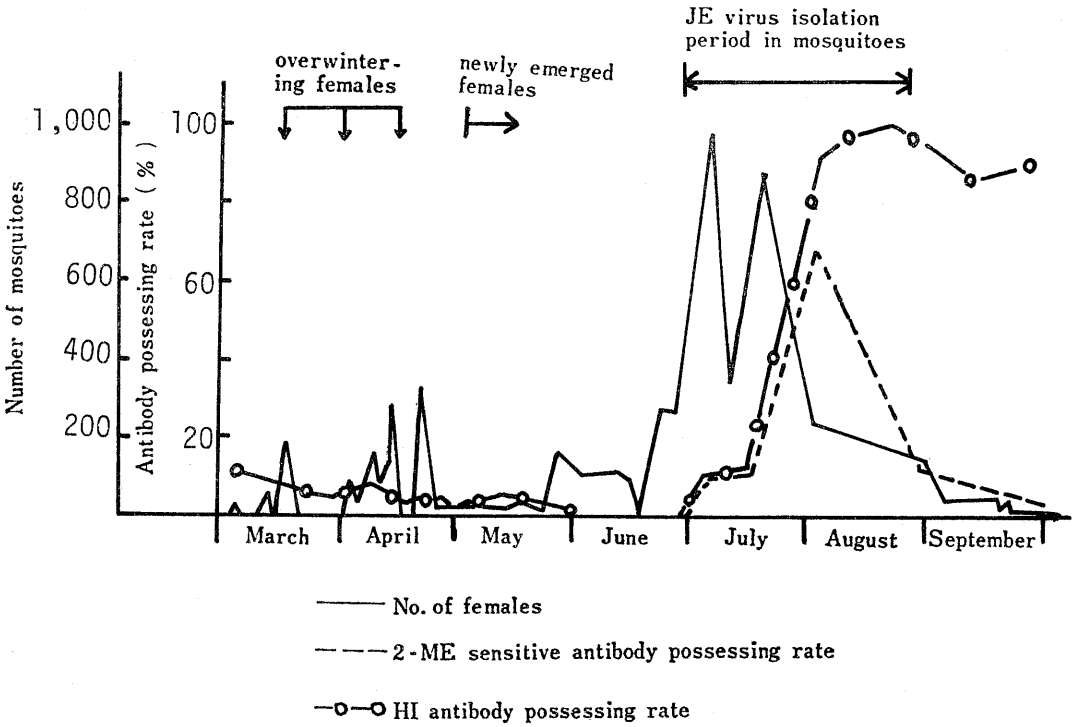


Fig. 3. Relation between antibody rising in swine sera and population of *C. tritaeniorhynchus* mosquitoes in Nagasaki area, 1966.

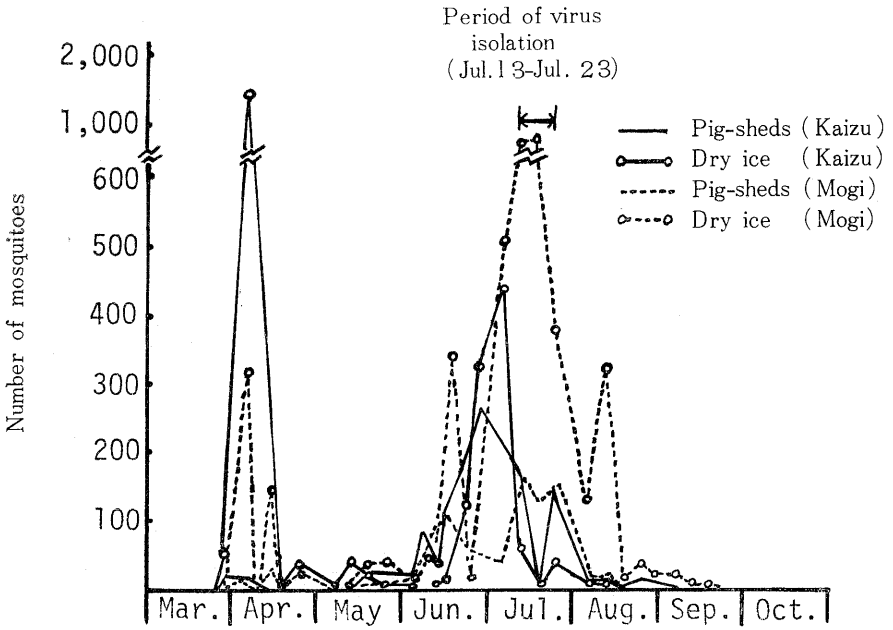


Fig. 4. Seasonal prevalence of *Culex tritaeniorhynchus* mosquitoes collected in the fields or at the pig-sheds in Nagasaki area and the period of JE virus isolation in 1971.

(3) To search the overwintering of JE virus.

(i) Investigation on the JE virus infection of common snakes in Japan

An attempt to isolate the virus from sera of 305 wild snakes captured in the field in 1969 had been unsuccessful, however, HI antibody was found in six of 207 snakes at low titer. Sixty-four snakes were inoculated with JE virus at various temperatures in an attempt to see the virus recovery from their blood at intervals of one to seven days during the experiment. In only five cases, the virus was recovered once for all on the third day to sixth day after inoculation. HI antibody was found in only one of 60 snakes at low titer and no response of neutralization antibody could be observed. These findings seem to imply that the common wild snakes in Japan are little susceptible to JE virus and they are in turn considered to play a minor role in the epidemiology of JE (Mifune et al. 1969) (Table 2 and Table 3).

(ii) Experimental infection of hibernated female *Culex tritaeniorhynchus* mosquitoes with JE virus.

Table 1. Virus Isolation from Vector Mosquitoes in Nagasaki Area

Year	Period of virus isolation		Period of virus Dissemination (days)
	fron	to	
1964	Jun. 8	Aug. 7	61
1965	May. 30	Sept. 6	100
1966	Jun. 24	Aug. 27	65
1967	Jul. 23	Jul. 27	35
1968	Jul. 18	Aug. 21	35
1969	Aug. 1	Aug. 21	21
1970	Jul. 19	Aug. 16	28
1971	Jul. 13	Jul. 27	15
1972	Aug. 16	Sept. 9	25
1973	Jul. 10	Jul. 24	15
1974	Jul. 29	Aug. 12	15

Table 2. JE virus isolation and HI antibody detection in the sera of various snakes

Species	virus isolation		HI test against JE virus	
	No. of sera tested	No. of virus isolated	No. of sera tested	No. of sera positive
Rhabdophis tigrinus tigirnus	213	0	186	3(*10,10,40)
Elaphe climacophora	28	0	24	0
Elaphe quadriuirgata	49	0	47	2(10,20)
Natris vibakari	6	0	4	0
Elaphe conspicillata	4	0	4	0
Agkistrodon hayls	5	0	5	1(10)
Total	305	0	270	6

* The figures in parentheses show each HI antibody titer expressed by the reciprocal of serum dilution.

(a) Adult female vector mosquitoes were infected with JE virus in the last part of October, 1964. They were exposed to the winter conditions at different places. The mortality was very high, however, a small number of them could survive for so long as 168 days over the winter until the latter part of March, 1965. The transmission of JE virus in only an infected hibernated female mosquito to the susceptible swine was successful. From this study, the possibility of the overwintering of JE virus in infected vector mosquitoes in nature was suggested (Mifune, 1965).

(b) Experimental infection of vector mosquitoes rared in Biotron with JE virus.

In preliminary experiment in which infected mosquitoes were kept in conventional incubators, it was observed that the virus growth in infected mosquitoes was prolonged in time at lower temperatures. The virus could still replicate even when the infected mosquitoes were kept at 15°C throughout the experiment. The mosquitoes for under-described experiments were rared in the indoor insectary biotron in which the astronomical day length, temperature and humidity were automatically regulated to change by daily cycle. Adult female mosquitoes which emerged under various conditions were infected with JE virus by allowing them to bite on infected chickens or by heeding the mixture of rabbit blood and virus suspension. The virus in mosquitoes which emerged and infected under various conditions had began to replicate from the third to seventh day after infection and reached the

Table 3. Experimental infection of snakes with JE virus

Species	No. of snakes JE virus inoculated	Tempe-rature exposed	Inoculum dosis of virus	Observa-tion periode (days)	No. of snakes JE virus recovered	No. of sera turned to HI antibody rising / No. of sera tested	No. of aera turned to NT antibody rising / No. of sera tested
R. tigrinus tigrinus	11	room temp.	$10^{2\cdot5-7\cdot5}$	29–36	2	0/11	0/ 1
	8	25°C	$10^{3.5-5.5}$	31	0	0/ 8	
	7	15°C	$10^{3.5-5.5}$	37	0	1/ 7	0/ 3
	4*	6°C	$10^{5.0}$	35	0	0/ 2	
E. quadriuirgata	5	room temp.	$10^{3.5-7.5}$	30–35	1	0/ 8	0/ 1
	8	25°C	$10^{3.5-6.5}$	31–34	0	0/ 5	0/ 5
	2	15°C	$10^{3.5}$	37	0	0/ 2	
	3**	6°C	$10^{5.0}$	32	0	0/ 2	
E. dimacophora	4	room temp.	$10^{3.5-7.5}$	29–40	0	0/ 4	0/ 3
	5	25°C	$10^{3.0-6.5}$	31–34	0	0/ 5	0/ 2
	2***	6°C	$10^{5.0}$	32	0	0/ 1	
E. conspicillata	2	room temp.	$10^{5.5-7.5}$	30	1	0/ 2	
	1	25°C	$10^{4.5}$	34	0	0/ 1	
N. uibakari	2	room tump.	$10^{7.5}$	17	1	0/ 2	
Total	64				5	1/60	0/15

* Two of 4 died during the exposure at 6°C
** One of 3 died during the exposure at 6°C
*** One of 2 died during the exposure at 6°C

maximum titer on the seventh to fifteenth day. However, the virus titer in case of the mosquitoes emerged under the environments of after middle part of October to November was constantly much lower than that in infected case of the mosquitoes emerged in the conditions of before the end of September (Shichijo et al. 1972) (Fig. 5 and Fig. 6).

(iii) An attempt to isolate the virus from hibernated female mosquitoes of of *Culex tritaeniorhynchus*.

The hibernated female vector mosquitoes were caught by the mothod of evaporation of dry ice in the field in early spring from 1965 to 1973. The virus isolation from 80,153 mosquitoes in total was examined but in vain (Table 4), (Hayashi et al. 1973).

(iv) What does it mean isolate no virus from hibernated female vector mosquitoes caught in the field in Nagasaki area during nine years.

It is reasonably presumed that if vector mosquitoes are experimentally infected with JE virus, the virus should persist for a long time and should be able to multiply in the mosquitoes under the enviroment of lower temperature even at 15°C.

Nevertheless, no virus could be isolated from hibernated female vector mosquitoes caught in the field in Nagasaki area. On the basis of result obtained, it was only to consider that the vector mosquitoes had not been infected with JE virus before their hibernation. In fact, there was no evidence to support the infection from the studies on the follicular development and feeding activity of the females of vector mosquitoes (Oda et al. 1973).

3. How does JE virus disseminate in whole Japan ?

It had been demonstrated from the reults of virus isolation from vector mosquitoes

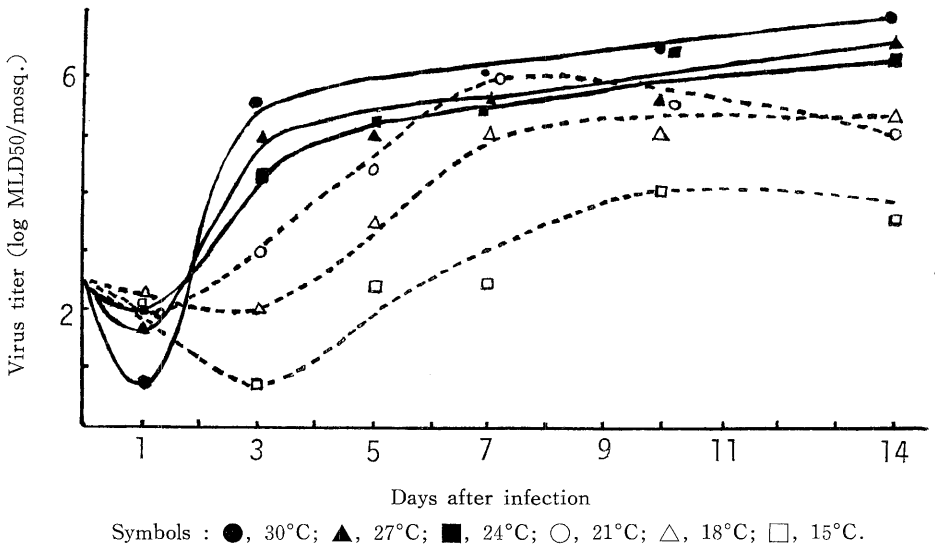
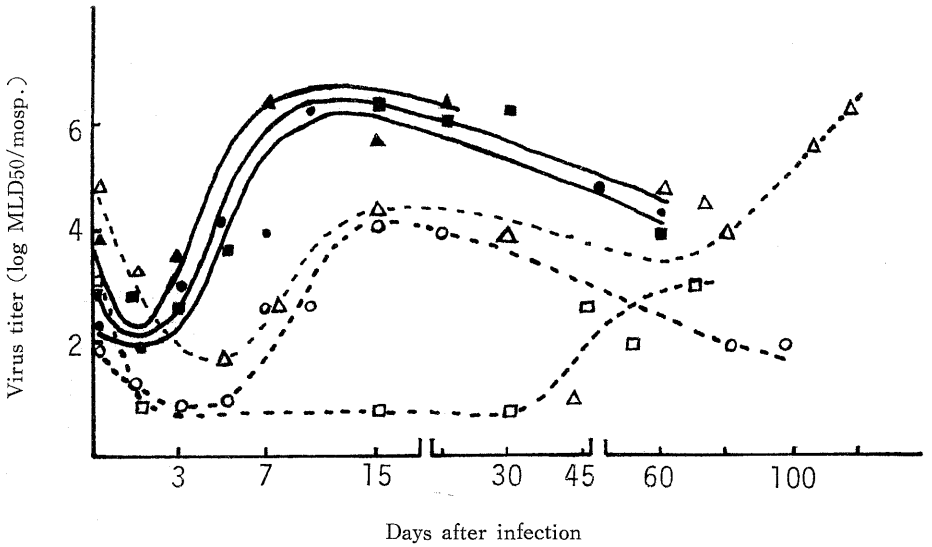


Fig. 5. Virus concentration of infected mosquitoes after incubation of various constant temperature.



Symbols indicate the period of emergene of female mosquitoes: middle part of August (●——●), latter part of August (▲——▲), middle part of September (■——■), latter part of September (○……○), latter part of October (Exp. 1. △……△, Exp. 2. □……□).

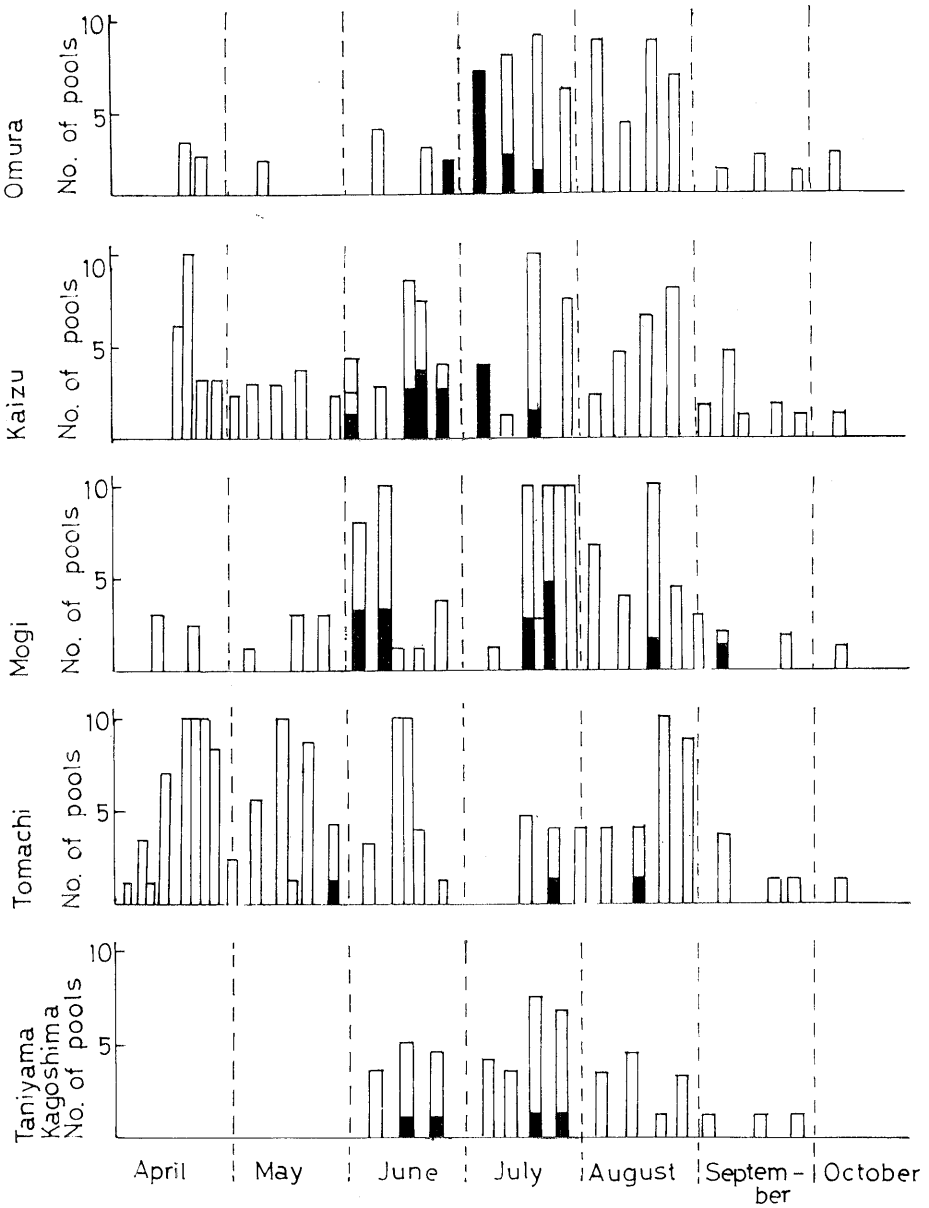
Fig. 6. Virus concentration in infected mosquitoes reared in various conditions of biotron.

Table 4. Attempts to isolate JE virus from overwintered *C. tritaeniorhynchus*

Year	March	April	May	Total	Methods used for virus isolation
1965	0	15,547	533	16,080	3-4 day old suckling mice, ic
1966	1,432	18,735	14,805	34,972	3-4 day old suckling mice, ic 10day old chicke mbryo, amniotic cavity
1967	1,517	12,492	0	14,009	3-4 day old sucklingmice, ic biting experiment, subcutaneous inoculation of mosquito suspension into susceptible pigs
1968	56	606	186	848	3-4 day SMB
1969	49	7,559	104	7,712	SMB
1970	0	1,082	143	1,225	SMB
1971	217	2,322	205	2,744	SMB
1972	22	1,935	0	1,957	SMB
1973	0	372	234	606	SMB
Total	3,293	60,650	16,210	80,153	

Remarks : ic …… intracerebral inoculation
SMB …… suckling mouse baby inoculation

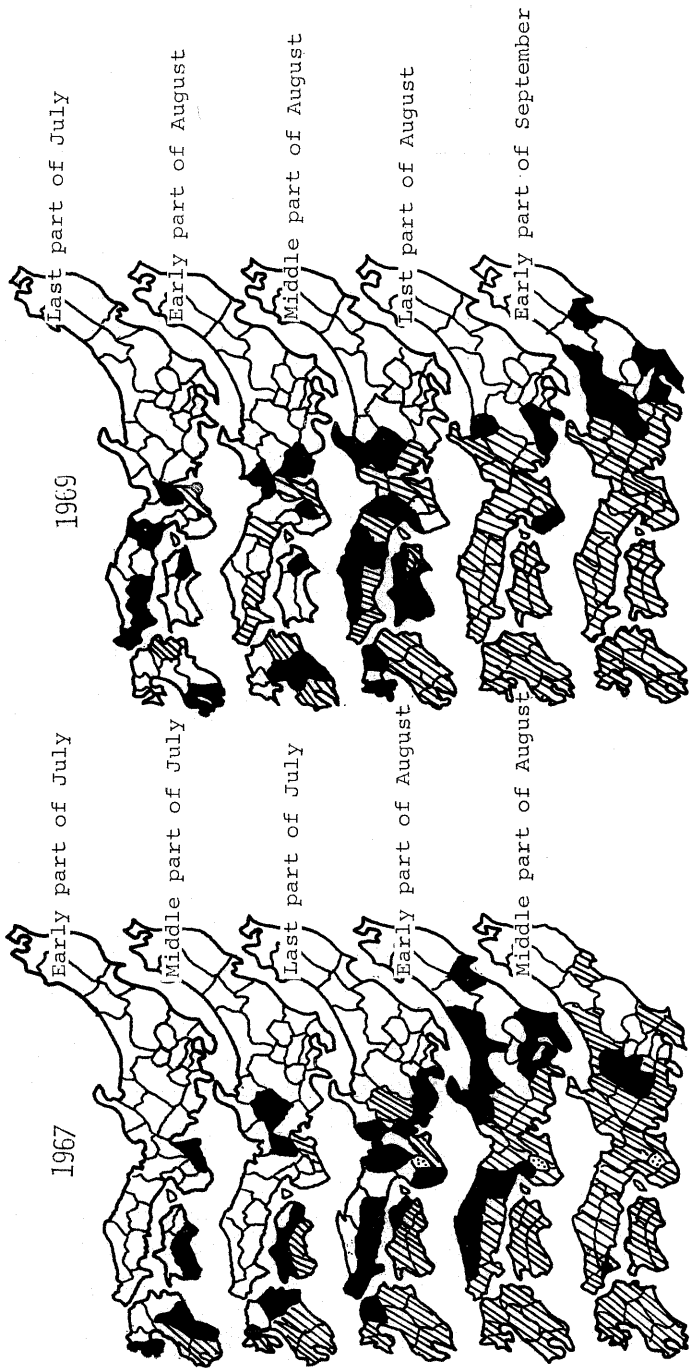
that, in epidemic season, the time and period of virus dissemination in the field had been varied by place (Fig. 7). However, the information on virus dissemination has been generally obtained from the results of the detection of HI antibody in slaughtered



The white sticks indicate the number of pools which could not isolate JE virus from *Culex tritaeniorhynchus* mosquitoes and the black sticks indicate the number of pools which could isolate the virus from them.

Fig. 7. Isoation of JE viruses from moiquitos in five different places in Nagasaki and Kagoshima prefectures in 1965.

swine sera. Thus, the information showing HI antibody confirmed at a level of 50 percent or 100 percent in the sera of slaughtered swine in the main islands of Japan has been announced monthly since 1965 by the Ministry of Health and Welfare of Japan. As seen in the representative results in Fig. 8a (1967) and Fig. 8b(1969), it is noted



Remarks: The shaded and black portion in the map are shown that the HI antibody possessing rate reached at a level of 50 percent before and after July respectively.

Fig. 8. Distribution of HI antibody possessing rate reached at a level of 50 percent in the sera of slaughtered pigs in main island of Japan in 1969 and 1969.

that the virus dissemination in the earliest stage of the epidemic season has been observed in the south part of Kyushu island (Kagoshima), the southern part of Shikoku and Mie area in the middle part of the main island of Japan. These findings indicate that the focuses of the virus dissemination might have appeared in these areas in the early epidemic season. If this proves to be the case, the question arises as to how it happen to induce the focuses in Japan. Eventually, this question involves the problems of overwintering of the virus in the main islands of Japan located in the temperate zone. On the point of view of the localized situation for the JE virus ecology to be proved, it will be necessary to investigate comparatively on the virus dissemination in the main island of Okinawa and Amami island located in the subtropical zone, with reference to that in Nagasaki area (Fig. 9).

4. Characteristics of the virus dissemination in the main island of Okinawa.

During the last eleven years, it was found that the percentage of HI antibody possess in swine sera had been increased, and simultaneously, the virus isolation had been made in Okinawa two or three months earlier than that in Nagasaki area (Table 5, Fig. 10 and 11). It was also found that the period for the virus dissemination in nature in

Fig. 9. Map of Japan, Korea and a part of China.

Table 5. First detection of 2ME sensitive HI antibody in pigs sera and first virus isolation from *C. tritaeniorhynchus* mosquitoes in Nagasaki and Okinawa by year.

Year	Area	Date of detection of HI antibody (2ME)	Virus isolation	Intervals of HI antibody rising between Nagasaki and Okinawa
1966	Okinawa	Apr. 11	ND	
	Nagasaki	Jul. 4	Jun. 24	84
1967	Okinawa	May 2	ND	
	Nagasaki	Jun. 22	Jun. 23	51
1968	Okinawa	May 15	ND	
	Nagasaki	Jul. 20	Jul. 23	66
1969	Okinawa	May 7	Apr. 16	
	Nagasaki	Jul. 24	Aug. 1	78
1970	Okinawa	Jun. 9	ND	
	Nagasaki	Aug. 7	Jul. 19	59
1971	Okinawa	May 4	May 11	
	Nagasaki	Jul. 6	Jul. 13	63
1972	Okinawa	Jun. 27	Jul. 11	
	Nagasaki	Sept. 5	Aug. 16	66
1973	Okinawa	May 23	Jun. 6	
	Nagasaki	Jul. 24	Jul. 10	63
1974	Okinawa	May 24	May 13	
	Nagasaki	Aug. 6	Jul. 29	78

ND : Not done

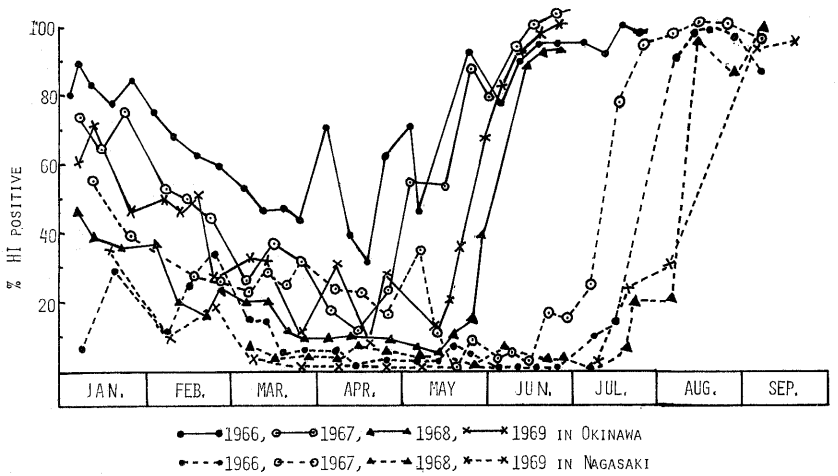


Fig. 10. Comparative investigation on HI antibody rising in pigs sera in Nagasaki area and Okinawa island from 1966 to 1969.

Okinawa had been prolonged compared to that in Nagasaki area.

5. Investigation on the ecology of JE virus in the interepidemic season in the main island of Okinawa.

An attempt to isolate the virus from resting vector mosquitoes caught in the field from November to next March since 1970 has been unsuccessful. However, the virus isolation from slaughtered swine sera had been made every year in the early part of April or at the latest in the middle part of May. In connection with the transmission of the virus to susceptible swine in the interepidemic season, it was a noteworthy fact that the 2ME sensitive antibody had been detected in January or February in 1968 to 1969.

6. Investigation on the ecology of JE virus in Amami island.

(1) Successful isolation of JE virus from hibernated female mosquitoes of *Culex tritaeniorhynchus* caught in the field in Konia village, the southern part of Amami island.

Four strains identified as JE virus were isolated from 8 pools of 1,083 hibernated female vector mosquitoes collected during the early to the middle part of February, 1973 and were transported by airlift to our laboratory from Amami island. This was the first case in whole Japan and was of great signigicance in connection with a long-term persistence of the virus in the interepidemic season in Amami island (Fig. 12, Fig. 13 and Fig. 14).

(2) Do the female mosquitoes of *Culex tritaeniorhynchus* carry different characteristics in the physiological nature in Nagasaki area, main island of Okinawa and Amami island ?

The development of follices, the blood feeding activity and the incidence of gonotrophic dissociation of the female vector mosquitoes which were rared in biotron or caught in the cowsheds in Nagasaki area had been examined by Oda et al. (1973). They have stated that: (i) most of the females entering into hibernation in autumn are unfed and nulliparous ; (ii) even if the gonotrophic dissociation in females of vector mosquitoes are observed in autumn, the females which experienced the gonotrophic dissociation must be extremely minor in number in the overwintering population; and (iii) the gonotrophic dissociation does not play any significant role the overwintering ecology of vector mosquitoes and there is

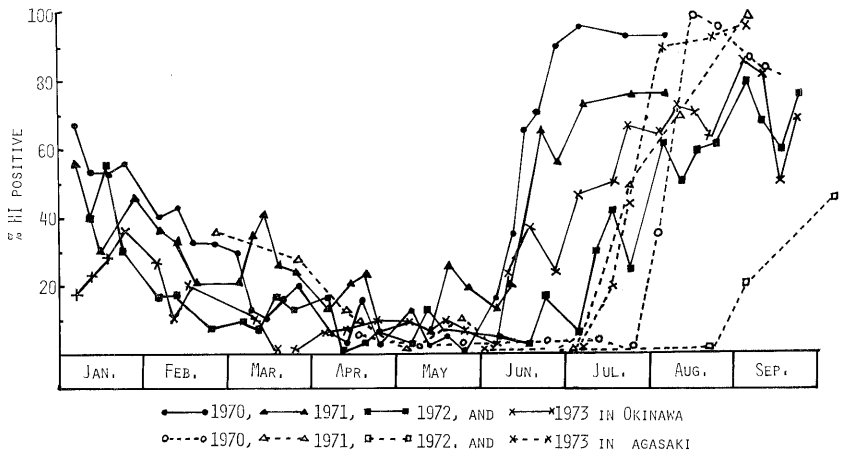


Fig. 11. Comparative investigation on HI antibody rising in pigs sera in Nagasaki area and Okinawa island from 197 to 1973.

no possibility for the persistence of the virus in vector mosquitoes over the winter so far as the northern part of Kyushu island including Nagasaki area is concerned (Oda et al. 1973). On the other hand, it was found that, during interepidemic season, the resting state of vector mosquitoes is usually variable by day in Okinawa and Amami islands. Consequently, the mosquitoes are stimulated the feeding activity by warm temperature even in winter period.

(3) Characteristics of the virus dissemination in Amami island.

As stated above, the virus had been isolated from vector mosquitoes and, subsequently, 2ME sensitive antibody in swine sera was detected in February, 1973. After that, the continuous virus infection in vector mosquitoes and swine was observed clearly through

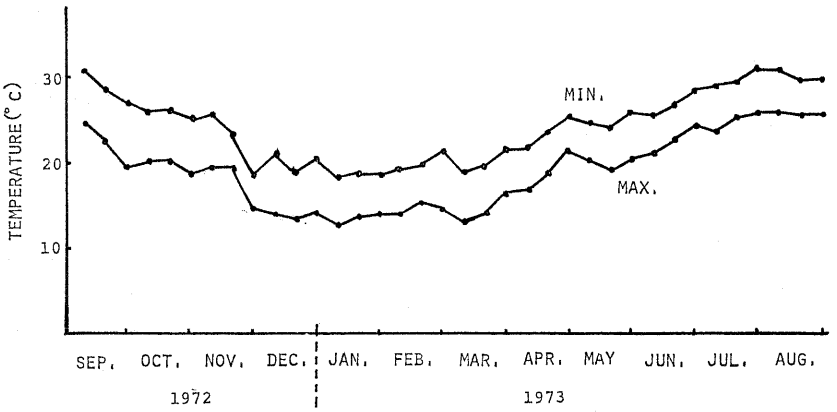


Fig. 12. Average temperatures in survey area (Amami, 1972–1973).

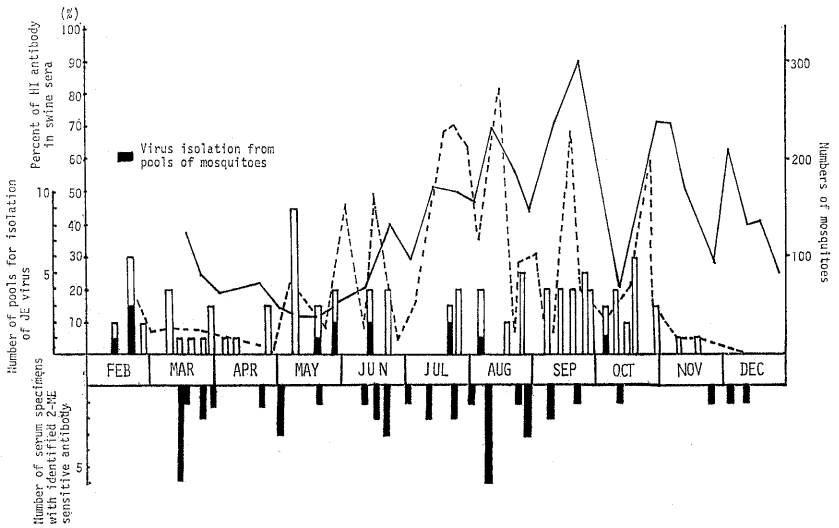


Fig. 13. Correlation between the infection of *Culex tritaeniorhynchus* mosquitoes and swine with JE virus and seasonal prevalence of the mosquitoes in Amami island in 1973.

the year. In 1974, however, the virus isolation from hibernated female vector mosquitoes was not performed early spring and but in July. This evidence suggested the interruption of the virus in the cycle of infection between vector mosquitoes and swine in the interepidemic season, and the virus might have been carried from elsewhere to the survey area in Amami island. These are remarkable findings in connection with the problems on the overwintering of JE virus in Japan.

(4) The flight over the ocean of female mosquitoes of *Culex tritaniorhynchus*.

Vector mosquitoes could be captured with light traps set up on the ship in the nearly middle part of the ocean between Kagoshima port and Amami island during the time from the midnight on 24th to very early in the morning on 25th Jnly, 1973. This finding suggest that vector mosquitoes might be transported with the wind over the ocean.

(5) Other animals and insects which may be concerned in the cycle of infection with JE virus in the field of Amami island.

Although it cannot be said definitely, it may be able to presume that no animals and insects except vector mosquitoes and swine are infected with the virus persistently. Even though the specimens examined were relatively by small in number, an attempt to isolated the virus from wild boars, wild rabbits and *Trimeresurus flavoviridis* had been unsuccessful, and no HI antibody could be detected in their sera. In addition, no virus could be isolated from about 500 ticks of such species as *Amblyomma testudinarium*, *Haemaphysalis formosensis* and *hystricis* caught in the mountain.

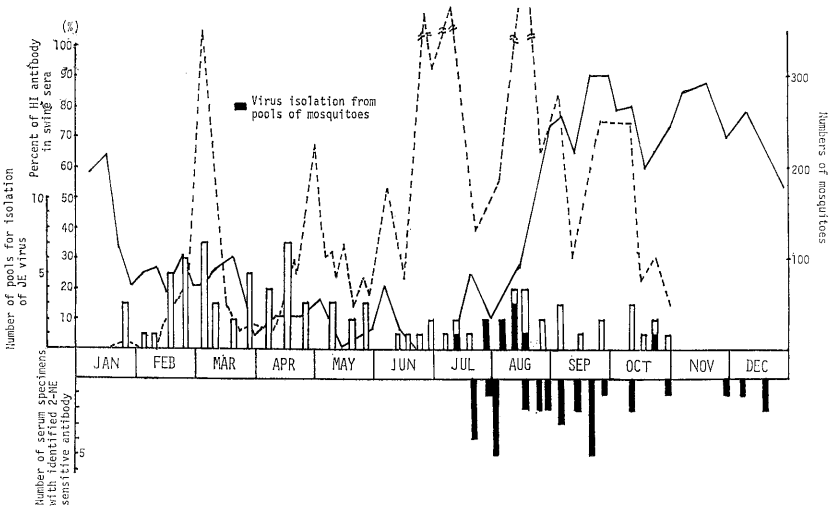


Fig. 14. Correlation between the infection of *Culex tritoeniorhynchus* mosquitoes and swine with JE virus and seasonal prevalence of the mosquitoes in Amami island in 1974.

DISCUSSION AND SUMMARY

As stated above, the term and the dulation for the virus dissemination in nature in Nagasaki area have become to be much later and shorter in every epidemic season since 1969. In other words, the period of the interepidemic season has been prolonged since 1969 compared to that before 1968. It is considered to imply that the focuses in the field in the early epidemic season might be decreased in number and in scale for the sequential amplification of the virus.

The climate in Japan has changed in a certain regularity through the year and it has not markedly varied since the ecological study of JE virus in Nagasaki area in 1964. Therefore, supposing the persistence of the virus somewhere Japan, the virus dissemination might in nature happen to appear earlier than the beginning of usual term in the epidemic season in every year. However, in fact, the virus isolation from vector mosquitoes has been made in Nagasaki area after the middle part of July since 1968.

As shown in Table 6, in the main island of Okinawa, the virus dissemination has usually been detected two or three months earlier than that in Nagasaki area since 1969. However, it was recognized in some years that the infection of vector mosquitoes and susceptible swine was interrupted for a short time in winter, namely from November to next March.

In Amami island, the vector mosquito and swine infection was investigated without interruption in 1973. However, the virus dissemination in nature occurred in the middle part of July in 1974. It can be concluded that the localized persistence of the virus might be preserved under the suitable conditions, and on the other hand, when it was interrupted in a certain year, the virus might be carried again from elsewhere to the survey area. These new findings are important informations for the studies on the ecology of JE virus in Japan.

The vector mosquitoes were captured in the ocean between the port of Kagoshima and Amami island in July 1973. Similar results had been obtained in the East China Sea and the Pacific Ocean as stated by Asahina (1970).

On the other hand, it was usually observed that migrating butterflies had been transported over the ocean and reached to Kagoshima area, the southern part of Kyushu, in the late spring or the early summer every year (Fukuda, 1970). It can be considered that they are transported with the wind blowing strongly into certain southern area of Japan. Taking these informations into consideration, it may be possible to propose an speculation that the mosquitoes infected with JE virus will be transported with the wind into the southern part of Japan from a certain epidemic area.

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長崎地方，沖縄及び奄美大島における日本脳炎ウイルスの生態

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1964年から10年間に亘って長崎地方における日本脳炎（日脳）ウイルスの生態について調査研究を行ってきた．1969年以後は沖縄本土と長崎地方の日脳ウイルスの撒布の地域特異性を検討した．1973年以後は奄美大島における調査を併行して行い日脳ウイルスの越年に関する問題を追究した．長崎地方では流行期における日脳ウイルスの撒布は1968年以前では60日ないし100日の長期間に亘って観察されたが，1969年以後は著しく短期間となり約2週間前後である．このような自然界における日脳ウイルスの撒布の変化と平行して人の日脳流行も減少してきた．過去10年間，長崎地方で早春に捕集した越年雌成虫コガタアカイエカ80,153個体からのウイルス分離は不成功に終っている．長崎地方では越年雌成虫は前年秋に吸血した証拠に乏しいことから，長崎地方ではウイルスを保持して越年する可能性が考えられないことが判かった．沖縄本土では野外における日脳ウイルスの撒布は長崎地方より2ケ月ないし3ケ月も早い．しかし，11月から翌早春3月までは野外蚊ないし豚のウイルス血症からのウイルスの分離は出来ない．即ち，期間は短くても流行閑期を認めることが出来る．この期間に2ME感受性抗体が検出されることがあるが，この事実は沖縄本土における日脳ウイルスの生存環を考える上に重要な資料である．奄美大島では1973年2月に豚舎で捕集した越年コガタアカイエカ1083個体の8プールから4株の日脳ウイルスを分離した．また，屠殺豚の血清中の2ME感受性抗体も同時に併行して検出された．このことは我国で始めての経験であって極めて重要な所見である．しかし，1974年には蚊からのウイルス分離は7月になってようやく可能であって，流行閑期においてウイルスの感染環が中絶したことを意味していると共に流行期にウイルスの持ち込みがあったものと考えてよい．即ち，奄美大島では蚊一豚の感染環は条件さえよければ年間を通じて保持されるが，その感染環は時に中断され，再びウイルスの持込みが行われるに違いないという特異な現象を示している．この現象は日本における日脳ウイルスの越冬を解明する上に極めて重要な所見として注目される．

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