Arbovirus infections in the mosquitoes of Fukuoka area, Kyushu, Japan

3. Natural infection of mosquitoes with Japanese encephalitis virus in the period from 1963 to 1972

Hideho YAMAMOTO*

Environmental Biology Section, Fukuoka Environmental

Research Center, Fukuoka 818-01, Japan

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Abstract: The present paper summarizes the results of the linear studies on the natural infection of mosquitoes with Japanese encephalitis virus in Fukuoka area in the period from 1963 to 1972 when the epidemics of Japanese encephalitis were yearly prevalent among residents. Descriptions and discussions are given principally of the annual and local variations in infection pattern, infection rate and infection time of *Culex tritaeniorhynchus* mosquitoes.

There was a marked tendency to intensify the epidemics of Japanese encephalitis throughout Japan in the period from the middle 1950's to the middle 1960's. During the period, the epidemics of Japanese encephalitis broke out yearly in larger scales among residents in Fukuoka area, where the number of the overt cases with the disease attained to 343 (serologically confirmed cases 206) in the summer of 1966. This outbreak of the disease was the largest in epidemic size as far as experienced in Japan. The natural infection patterns of vector populations with Japanese encephalitis virus in such an intensive epidemic area will be of special note. In the present paper the results of the 1963 to 1972 studies in Fukuoka area are summarized. The epidemiological considerations will be given in the following paper of this series.

STUDY SITES

The study consisted of 2 series of the perennial observations on the natural infections of *Culex tritaeniorhynchus* mosquitoes with Japanese encephalitis virus (JEV) at the specified zones and additional works at various localities. One series of the perennial observations was

made at the study sites of the Kasuya district (Kasuya-gun) in the period from 1963 to 1967 (study series I), and the other series at the study sites of the Kanatake district (Fukuoka-city) in the period from 1968 to 1971 with an additional observation at a study site of Maebara (Itoshima-gun) in 1972 (study series II). All the study sites are shown in Fig. 1.

The study sites of the Kasuya district were placed at 3 villages, Uchihashi, Aé and Shime. Uchihashi and Aé are contiguous to each other, and thus designated at the Uchihashi-Aé study site. The villages lie on the plain area and are surrounded by vast rice fields. The Uchihashi-Aé study site included 5 sampling spots: 2 cattle sheds, a fowl shed and 2 pig sheds. The Shime study site included a single sampling spot, a pig shed.

Four study sites were set in the Kanatake district: Toji, Iimori, Nishiyama and Shika. The Kanatake district occupies the southwest suburban zone of Fukuoka-city along hills of 300 to 400 m altitude. The sampling spots placed at the respective study sites were: unspecified pig sheds in Toji, 2 pig sheds and a cattle shed in Iimori, 2 pig sheds and a cattle shed in Nishiyama, and a cow barn in Shika.

In the Maebara study site mosquito sampling was carried out in a pig shed and 2 cow barns.

Besides the study sites above referred to,

* 山本英徳：福岡県衛生公害センター環境生物課（〒818-01 福岡県筑紫郡太宰府町大字向佐野字迎田39）

temporal study sites were set at several localities for timely programs of study.

Mitsuzawa (Mii-gun) : in this study site, through-season mosquito catches were made in the pig farms of the Fukuoka Prefectural Zootechnical Station in 1966 in addition to the Kasuya and Kanatake studies.

Onga (Onga-gun), Honami (Iizuka-city), Minagi (Amagi-city) and Sedaka (Yamato-gun) : in these study sites, one-night mosquito catches were once made in 1967. In all the study sites sampling was carried out in pig sheds.

Fig. 1 A map showing study sites

Double circles signify the specified study sites at which perennial observations were made, open circles the temporal study sites at which one-night mosquito catches or single-year programs of study were carried out, and the shaded region Fukuoka-city area.

MATERIALS AND METHODS

The natural infection of mosquitoes with JEV was evidenced by means of virus isolation technique with field-caught mosquitoes. Mosquito catches were made at an interval of 1 week throughout the season from April or May to October. Based on the results of the preparatory studies (Yamamoto, 1978), only non-engorged females of the mosquitoes which were caught with aspirators in animal sheds were provided for virus isolation attempts. In mass sorting of the mosquitoes, a close attention was paid to discriminating between *C. tritaeniorhynchus* Giles and *Culex pseudovishnui* Colless. As shown in a former note (Yamamoto, 1979), the relative abundance of *C. pseudovishnui* to

C. tritaeniorhynchus attained to 0.7 to 0.8% on an average in Fukuoka area. The mosquito samples differed in number with study sites and were varied in pool size with study years and the purposes of studies. The number of mosquitoes in a pool was 200 only in the 1963 study, 100 throughout the 1964 to 1968 studies, and varied with the infection rates expected in the 1969 to 1972 studies in which it was 25 in minimized size and 50 or 100 in ordinary size. Infection rates of mosquito samples were compared among different populations or among different years on the basis of the infection rate of mosquito population (IRMP) proposed by Chiang and Reeves (1962).

Virus isolations were made in suckling mouse inoculation system with mosquito-tissue suspensions, and the isolates obtained were identified through hemagglutination (HA) and hemagglutination-inhibition (HI) tests. HA antigens of isolates were prepared by crude alkaline technique in the primary screening tests and by sucrose-acetone extraction technique in the final identification tests.

The details of the techniques for mosquito sampling, preparation of materials, virus isolation, identification of isolates, race of mice used and reference viruses were fully described in the previous papers (Yamamoto, 1978, 1980; Yamamoto and Manako, 1968, 1970). There were some amendments in contents of diluent for mosquito-tissue suspensions during the 10-year studies, and the contents of the diluent used in each study were shown in the 2nd paper of this series (Yamamoto, 1980).

RESULTS

1. Failure in detecting Japanese encephalitis virus from mosquitoes other than *Culex tritaeniorhynchus*

Table 1 summarizes the results of the virus isolation attempts with 5 prevalent species of mosquitoes during the 10-year studies. All successful isolations of viruses were made exclusively from the non-engorged females of *C. tritaeniorhynchus* : 244 isolates were finally determined as JEV, 9 as the viruses of Getah complex, and the remainder could not be identified. Eventually the mosquitoes other than *C. tritaeniorhynchus* did not yield any viral agent.

2. Natural infection patterns in *Culex tritaeniorhynchus* populations with Japanese encephalitis

Table 1 A summary of the 1963 to 1972 results of virus isolation attempts with 5 species of mosquitoes

Mosquito species	No. mosquitoes tested	No. mosquito pools	Viruses isolated		
			JE	Getah	Others
<i>Culex pipiens</i> complex	4,701	80	0	0	0
<i>Culex tritaeniorhynchus</i>	205,873	2,915	244	9	1
<i>Culex pseudovishnui</i>	665	28	0	0	0
<i>Culex whitmorei</i>	220	7	0	0	0
<i>Anopheles hyrcanus</i> group	2,050	32	0	0	0

Table 2 Results of Japanese encephalitis virus isolations from *Culex tritaeniorhynchus* mosquitoes in the study series I in Kasuya district (1963–1967)

Month	Sub-division*	Uchihashi-Aé								Shime	
		1963		1964		1965		1966		1967	
		<i>n</i>	JEV	<i>n</i>	JEV	<i>n</i>	JEV	<i>n</i>	JEV	<i>n</i>	JEV
May	III			36	0/1						
	IV	1	0/1	—	—	6	0/1				
June	I			—	—	118	0/2	175	0/2		
	II			26	0/1			122	0/2		
	III			—	—	483	0/6	170	0/3	310	0/4
	IV	13	0/1	240	1/3	151	0/3	217	0/3	471	0/5
July	I	3	0/1	783	2/9	516	2/7	745	4/8	700	1/7
	II	112	0/2	1,978	4/20	1,365	3/15	1,671	0/17	615	1/7
	III	563	0/6	2,245	1/23	1,879	6/20	2,000	3/20	1,400	3/14
	IV	1,545	1/9	1,977	1/20	2,000	1/20	2,000	8/20	2,000	4/20
Aug.	I	564	0/4	1,893	0/19	1,000	0/10	1,500	5/15	1,800	1/18
	II	2,484	9/13	1,514	0/16	1,973	0/20	1,900	2/19	1,900	0/19
	III	3,153	10/16	1,030	0/11	2,000	0/20	2,000	1/20	1,500	0/15
	IV	2,450	8/12	1,081	0/12	1,261	0/13	1,900	0/19		
Sept.	I	151	0/3	—	—	1,935	0/20	1,800	0/18		
	II	—	—	—	—	295	0/3	1,451	0/15		
	III	1,186	0/7	1,245	0/14	265	0/3	1,567	0/16		
	IV	337	0/3	304	0/3	72	0/2	500	0/5		
Oct.	I	10	1/1	—	—			493	0/5		
	II	3	0/1	60	0/1			59	0/2		
				3	0/1			31	0/2		
Total		12,575	29/80	14,415	9/154	15,319	12/165	20,301	23/211	12,596	10/128

Abbreviations *n* : the number of mosquitoes tested, JEV : the number of JEV-positive pools / the number of mosquito pools tested, — : no mosquito sample was obtained.

* A month is subdivided into I (1st–8th day), II (9–16th day), III (17–24th day) and IV (25–30th or 31st day).

litis virus

The full results of the 1963 to 1972 studies are set out in Tables 2 and 3. For the detailed data of the 1963 to 1966 studies, readers are referred to Yamamoto *et al.* (1966, 1968) and Yamamoto and Manako (1968, 1970).

2-1. *Evidence for sporadical mosquito infections:* The JEV isolation attempts adduced a few lines of evidence for sporadical mosquito infections with JEV. Premonitory signs of mosquito infection were observed in 1966 and 1970. The clear evidence for mosquito infection was obtained 16 days before the onset of the concentrative mosquito infection (*cf.* 2-2) in 1966 and 14 days before that in 1970. Moreover, the earliest isolation of JEV at Iimori in 1968 may have adduced the evidence similar to the above.

On the other hand, there was the evidence for sporadical mosquito infection in late season

in 1963: 10 mosquitoes were caught on 26 September 1963 when it was 5 weeks after the concentrative mosquito infection was over, and the single pool of the mosquitoes yielded an isolate of JEV. This is the latest field evidence for JEV infection of mosquitoes as far as in the Japanese Main Islands, and thus deserves a notice in the respect that the infected mosquitoes were detected at a long interval after pig-mosquito cycles had been suppressed.

2-2. *Evidence for concentrative mosquito infections:* Tables 2 and 3 show that the mosquito infection was concentrically intensified in a period of mid-summer every year, and that the infected populations of *C. tritaeniorhynchus* sustained their infection rates above a detectable level during the period. The mosquito infection yearly proven in a serial pattern is thus referred to as the "concentrative mosquito in-

Table 3 Results of Japanese encephalitis virus isolations from *Culex tritaeniorhynchus* mosquitoes in the study series II in Kanatake district and Maebara (1968-1972)

Month	Subdivision*	Kanatake								Maebara	
		Iimori 1968		Nishiyama 1969		Nishiyama 1970		Shika 1971		1972	
		<i>n</i>	JEV	<i>n</i>	JEV	<i>n</i>	JEV	<i>n</i>	JEV	<i>n</i>	JEV
June	I			97	0/5						
	II	331	0/9	37	0/4	28	0/1				
	III	124	0/9	259	0/11	135	0/6				
	IV	781	0/8	536	0/11	62	0/3			172	0/7
July	I	3,128	0/25	2,272	0/23	639 1,986	0/13 0/40	1,581 2,000	0/64 0/80	700	0/14
	II	555	0/7	2,391	0/24	1,480	0/30	2,000	1/40	613	0/12
	III	1,593	0/18	1,053 2,021	0/21 0/21	2,000	1/40	2,000	5/20	2,000	0/20
	IV	3,395	0/34	2,646	0/27	2,000	0/40	875	4/35	1,850	0/37
Aug.	I	600	1/6	2,000	0/40	1,000	1/40	350	1/14	1,692	0/17
	II	970 1,132	0/10 6/12	1,150	6/23	975	12/39	1,750	1/35	600	0/12
	III	722	2/8	750	5/30	1,400	3/28	1,800	0/36	203 456	1/4 1/10
	IV	110	0/2	1,000	4/20	900	0/18	1,400	0/28	600	3/12
Sept.	I	683	1/7	1,500	2/30	—	—	500	0/20	413	4/9
	II	823	0/9	275	0/11	350	0/7	400	0/16	—	—
	III	631	0/7	893	0/18			247	0/10	30	0/1
	IV			11	0/1						
Oct.	I			7	0/1						
Total		15,578	10/171	18,898	17/321	12,955	17/305	14,903	12/398	9,329	9/155

Abbreviations are the same as in Table 2.

* See Table 2.

fection" in contrast to the sporadical mosquito infections referred to the above. A time series of IRMP's of weekly samples may probably indicate the progressive course of the concentrative mosquito infection. Therefore, yearly time series of IRMP's are arranged in Fig. 2 to facilitate a comparison in pattern among different years. The concentrative mosquito infection continued yearly for a duration of 4 to 5 weeks, and rarely down to the 6th weeks. In fact, the year to year variations in the period of the concentrative mosquito infection were mostly in a range of a week. The concentrative mosquito infections expired within the period, though the mosquito populations remained their sizes enough. This fact was best demonstrated in the 1964, 1965 and 1966 studies (Yamamoto and Manako, 1968, 1970).

The time series of IRMP's showed a salient peak in the 2nd week and rapid falls in the subsequent weeks in a typical pattern. The salient peak possibly signifies the outbreak of large-scaled amplification of JEV in pig-mosquito cycles, and the rapid falls the dispersal and extinction of infected mosquitoes and also the dilution out of them with the uninfected mosquitoes of subsequent generations. The results could be simulated to the typical pattern in the study ser. I except for the 1963 and 1967 results, while they showed considerable irregularities in the study ser. II.

Study series I (1963–1967) : The 1963 result was very unique in pattern, keeping IRMP high up to the 4th week. This pattern may be due to the larger size of mosquito pools (200 mosquitoes in a pool). The 1964 and 1965 results may have failed in detecting the evidence for the low-rate mosquito infection in the 1st week. The failure may be attributed to smaller sizes of the samples examined. In both the years *C. tritaeniorhynchus* populations remained to be grown and only small numbers of the mosquitoes were provided for the studies in the early summer. If this reasoning had been real, the concentrative mosquito infections in 1964 and 1965 would have been commenced in the middle and late June, respectively. The 1966 result showed a typical pattern in time series of IRMP's with the enough evidence for the premonitory infection of the mosquitoes. In 1967 the mosquito infection pattern kept its IRMP low during the period and did not reveal a salient peak infection.

Fig. 2 Time series of infection rates (%) in the concentrative infection of *Culex tritaeniorhynchus* mosquitoes with Japanese encephalitis virus, the 1963 to 1972 results in 2 study series. Yearly time series are arranged irrespective of the time of onset and date, and infection rates are expressed by week.

Study series II (1968–1972) : The concentrative mosquito infections broke out earlier than the peak population of the mosquitoes only in 1971 and in varied times after the peak population in the other years. The 1968 mosquito infection was interrupted in the 4th week, and the 1970 mosquito infection could not be detected in the 4th and 5th weeks. Undoubtedly, these discontinuities were due to the scarcity of mosquito samples. Extreme irregularities were observed in pattern of mosquito infection in 1972. The time series of IRMP's showed a depression in the 2nd week and then a rising trend from the 3rd to the 4th week attaining the peak value (11.69%) in the last week.

Table 4 Comparisons in infection rate (‰) with Japanese encephalitis virus among *Culex tritaeniorhynchus* samples collected at different localities

Year	Locality	June		July				Aug.				Sept.				
		III	IV	I	II		III	IV	I	II		III	IV	I	II	
					Early	Late				Early	Late					
1967	Shime	0	0	1.54	1.54	2.41	2.23	0.57	0		0	0				
	Uchihashi-Aé							0								
	Kanatake (Otoishi)								0							
	Onga						1.62									
	Honami						4.05									
	Minagi						0									
	sedaka							0								
1968	Kanatake					0	0	0	1.82	0	6.91	2.87	0	1.54	0	
							0									
	Uchihashi-Aé							0			4.05	10.93	0	0	0	

Each pool contains 100 mosquitoes ($m=100$) in all the attempts referred to. For the subdivisions in a month, see the footnote of Table 2.

Fig. 3 A comparison in time and pattern of Japanese encephalitis virus infection of *Culex tritaeniorhynchus* mosquitoes among different localities of Fukuoka area

The patterns of mosquito infection are presented by time series of infection rates (‰) of mosquito populations. The subdivisions of a month are the same as in Table 2. A: a comparison among different study sites in 1966, Uchihashi-Aé (solid), Mitsuzawa (open) and Kanatake (Toji) (hatched). B: a comparison between 2 study

sites in 1969, Kanatake (Nishiyama) (solid) and Shime (open). The mosquito infection rates (‰) at the Shime study site in the 2nd subdivision of August were estimated in 2 series of the mosquito pools from the same sample. In one series the number of the mosquito pools tested was 12 ($n=12$) and each pool contained 25 mosquitoes ($m=25$), and in the other series n and m were 8 and 50, respectively.

2-3. *Local variations in the time and pattern of mosquito infection:* Through-season studies were simultaneously carried out at Uchihashi-Aé, Mitsuzawa and Kanatake (Toji) in 1966, and at Kanatake (Nishiyama) and Shime in 1969. As shown in Fig. 3, the studies showed an approximate uniformity in the outline of mosquito infection pattern among different localities. Additionally, rough comparisons were attempted between the serial samples from Shime and the one-night samples from 6 localities in 1967, and between the serial samples from Kanatake (Iimori and Nishiyama) and the scattered samples from Uchihashi-Aé in 1968. These comparative attempts showed also an approximate agreement in the time of peak infection (Table 4). The time lags of peak infection were within a week in all the attempts.

DISCUSSION

A large amount of evidence for natural infec-

tion with JEV has been obtained from *C. tritaeniorhynchus* in Japan. The successful isolations of JEV were also frequently made by various investigators from the field-caught specimens of *C. pipiens* complex (Mitamura *et al.*, 1939; Buescher *et al.*, 1959; Matsuyama *et al.*, 1960; Oya *et al.*, 1960; Hayashi *et al.*, 1966; Maeda *et al.*, 1978) and rarely by a few investigators from those of *C. pseudovishnui* (Hayashi *et al.*, 1966; Shichijo *et al.*, 1968), *Aedes albopictus* (Skuse) (Hurlbut and Nibley, 1964), *Aedes vexans* Meigen (Hayashi *et al.*, 1966; Shichijo *et al.*, 1968) and *Armigeres subalbatus* (Coquillett) (Shichijo *et al.*, 1968). Despite the above facts, the present attempts failed to adduce the evidence for the natural JEV infection of the mosquitoes other than *C. tritaeniorhynchus*. The failure may be ascribable to smaller sizes of the samples examined. If those mosquitoes rarely supply the evidence for the natural JEV infection, they are hardly responsible for the wide-spread dissemination of the virus in the area. A total of culicine mosquitoes except for *C. tritaeniorhynchus* formed only 1 to 2% in relative abundance (Yamamoto, 1971). Anopheline mosquitoes are known to be resistant to JEV infection, though the mosquitoes occupied 13 to 14% in relative abundance.

In *C. tritaeniorhynchus* populations, the natural infection with JEV was evidenced every year and continued for a period. In principle, cyclic phases may be recognized in a yearly pattern of mosquito infection with JEV, the premonitory and subsequent phases. The premonitory phase was a passing phenomenon and small in scale so that it was not always detected. The subsequent phase which is referred to as the concentrative mosquito infection in the present paper broke out about 2 weeks after the premonitory phase and maintained a detectable level for 4 to 5 weeks. These were in agreement with Konno *et al.* (1966) who found cyclic outbreaks in the JEV infections among swine, mosquito and human populations in Miyagi-prefecture in 1964.

The detection of infected mosquitoes in the late September 1963 would attach little importance to the problem of overwintering of JEV, because the JEV isolate may have come from the survivals of the mosquitoes which had been infected in the mid-summer (Harada *et al.*, 1967; Buei *et al.*, 1968). In such areas of Kanto and northward as the cycles of JEV infection became detectable in later season and con-

tinued to early autumn, infected mosquitoes were not so rarely detected even in September (Buescher *et al.*, 1959; Konno, 1967; Matsuyama *et al.*, 1968). Therefore, the finding should be interpreted as a matter different from the detection of infected mosquitoes in the autumn to winter months in the South Western Islands of Japan (Hurlbut and Nibley, 1964; Hayashi *et al.*, 1975, 1976).

The mosquito infection broke out in the increasing course of mosquito populations (in the late June to the late July) in the years of the study ser. I, but often in the decreasing course (in the middle to late August) in the years of the study ser.II. Annual differences in pattern and IRMP values may have a direct bearing on the time of onset of mosquito infection. Excepting the 1967 result, the pattern and IRMP values did not show any significant annual variations in the study ser. I, while they showed wide annual variations in the study ser.II. Although I cannot give full explanation to the peculiar pattern in the 1967 mosquito infection, it may have direct bearing upon the fact that JEV infection developed slowly also in swine populations in the same summer (Otsuka *et al.*, 1969). The mosquito infection in 1972 was most exceptional in pattern. This fact may have resulted from the late-summer onset of mosquito infection. From the studies on JEV multiplication (Gresser *et al.*, 1958a, 1958b; Takahashi, 1976) and gonotrophic cycles (Yajima, 1973) in *C. tritaeniorhynchus*, it seems likely that the larger the proportion of parous females in field-mosquito samples, the higher the JEV isolation rate. Harada *et al.* (1967) and Buei *et al.* (1968) demonstrated that the proportion of parous females enlarged with the progress of season, attaining to 60 to 80% in September. In this connection, JEV infection rates of field mosquito samples might be subject to certain variables according to the physiological age composition of mosquito populations rather than to the grade of JEV infection. The time series of IRMP values which showed a reasonable course in typical cases might also suggest that pig-mosquito cycles give rise to an extensive amplification of the virus once in an epidemic season.

Recent studies clarified a clear geographical variations in the time of JEV infection in pig-mosquito cycles. However, it is uncertain how far the distance gives rise to the geographical difference. If the JEV infections of mosquitoes

take on local varieties of time and pattern even within a given area, it is irrelevant to make an annual comparison between the series of studies which were established at different localities. As far as the present study is concerned, the attempts to approach this subject made it possible to conclude that spot to spot variations in JEV infection of mosquito populations were a little in time and pattern within the given area. In this respect, the attempts are in good agreement with Oya *et al.* (1963) who demonstrated that the mosquito infection became concurrently detectable at 3 sites of Gunma-prefecture in 1961, but not with Buescher *et al.* (1959) who stated that the time of occurrence of infected mosquitoes varied several weeks from place to place in the Kanto plain. The above finding would justify the conclusion that the differences in mosquito infection pattern between the study sers. I and II signify annual variations but not local variations, because Kasuya (Uchihashi-Aé and Shime) and Kanatake (Toji, Iimori, Nishiyama and Shika) were the main study areas in which the perennial studies in the 2 study series were made, respectively, and the through-season studies on the local variations of mosquito infection pattern were simultaneously carried out at the study sites of the 2 areas.

In conclusion, before 1968 the natural infections of mosquitoes with JEV had occurred in the increasing course of *C. tritaeniorhynchus* populations in the southwestern regions of Japan and in the decreasing course in the northeastern regions of the country. This mode of mosquito infection ascribed to the northeastward extension of JEV dissemination and had been immutable in the regions till 1967. As shown in a former paper (Yamamoto, 1971), the official data on the history of epidemics of Japanese encephalitis in Fukuoka area apparently showed that yearly JEV infections had taken place in the area also in the past years before the study was initiated. Because of this, JEV infections in the increasing course of mosquito populations were referred to as "western type" and those in the decreasing course as "eastern type" in a former paper (Yamamoto, 1968). It is thus particularly important to emphasize that there was a drastic change in Fukuoka area from the western type to the eastern type in the mode of mosquito infection. The fact will attach an importance to the epidemiology of Japanese encephalitis. There-

fore, the data on JEV infection among *C. tritaeniorhynchus* mosquitoes will be discussed in regard to the epidemic sizes of the disease in the following paper with the data on the seasonal curve of the mosquito populations.

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摘 要

福岡地方における蚊のアルボウイルス感染に関する研究

3. 1963～1972年間に於ける蚊の日本脳炎ウイルス自然感染

該当期間内に福岡地方に普通に産する 5 種の蚊の野外採集材料を用いて、日本脳炎ウイルス (JEV) 保有を蚊組織懸濁液の哺乳マウス脳内接種法により検索した結果、JEV 自然感染は、コガタアカイエカだけでは毎年高い頻度で証明されたが、他種の蚊では証明できなかった。コガタアカイエカにおける JEV 自然感染パターンを毎年の成績から類型化すると、典型的に

は、一過性の先がけ的な保毒現象の約 2 週間後に同蚊の集中的な保毒が 4～5 週間継続するといえた。またコガタアカイエカの JEV 保毒率は時として異常なパターンで推移したが、それは同蚊母集団の年齢構成の推移に関連すると考えられた。当地方におけるコガタアカイエカの JEV 自然感染は、1963～1967 年間では、同蚊個体群の季節的増加の過程において発生したのに対し、1968～1972 年間では、しばしばその減少過程において発生するようになった。このことは、生産される保毒蚊の量および保毒蚊のその後の動態と関連するため、疫学的に重要な意義があったと考えられる。なお、この継続研究は、1963～1967 年間は福岡県粕屋郡内で、1968～1972 年間では大部分福岡市金武地区内で実施されたが、この間に、両地域間において全期間調査を再度同時的に行った。その結果では、コガタアカイエカの JEV 自然感染の時期、様相は両地域間で大きな差はなかった。したがって、前記のことはコガタアカイエカの JEV 自然感染における年次変動と考えてよいであろう。