Problems on Overwintering of Japanese Encephalitis Virus in Japan

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Abstract : In the present time, there are some important problems of the virological studies on Japanese Encephalitis virus, especially as the difficult works on the stand point of ecological views, no verification on overwintering of viruses in the natural world and, moreover, supplier of the viruses to the majority of *Culex tritaeniorhynchus* mosquitoes prior to the epidmic season have been carried out untill now. Present paper are described that the works of pioneers concerning the ecology of Japanese Encephalitis virus are summarized and additionally from the results of authors' work, some future research plans are discussed.

As it is well-known, Japanese Encephalitis viruses have been isolated from the mosquitoes of *Culex tritaeniorhynchus* every summer prior to an epidemic of Japanese Encephalitis (hereinafter referred to as JE) among human being, and it has been verified that a large numbers of *Culex tritaeniorhynchus* are infected with JE viruses. However, in spite of the studies by many pioneers up to now, the activity of virus ceases during the period from late autumn to the next early spring and no information is available even on the ecology of the *Culex tritaeniorhynchus* mosquitoes.

The result of the large scale survey carried out by SCHERER et al. (1959) from 1952 through 1957 and their highly reliable detailed study presented some important findings on the ecology of JE virus especially on amplifier, but it still could not verify the cycle of virus infection during the winter. However, the conclusion is now deeply impressed in the mind of many investigators.

The work of the pioneers who pursued the ecology of JE virus in the natural world during an epidemic season-off is now again summarized as shown in Table 1 to help establish future research plans in this field.

First of all, estimation of the ecology of JE virus during the winter is classified into the "carried-in" theory and the "overwintering" theory.

The former theory is classified further more.

(1) Theory that JE virus is carried in by migratory birds: As seen in cases of Equine Encephalitis virus in the west and the east of North America and of Murry Valley virus in Australia, it is believed that JE virus is carried in by the simultaneous

Table 1. Summary of Japanese Encephalitis Virus Infection

Ⅰ. Theories that JE virus was "Carried-in"
(1) Carried in by migratory birds
Western Equine Encephalitis Virus and Eastern Equine Encephalitis Virus (North America)
Murry Valley Virus (Australia)
Sagiyama Virus (Japan/Scherer et al. (1957))
〃 〃 (Malay/Buescher et al. (1960)
Akabane Virus (Japan/Oya et al. (1959))
(2) Carried in parasitic arthropods adbered to migratory birds
Ⅱ. Theories of Overwintering of JE virus
(1) Overwintering of infected mosquitoes
(2) Egg-passage of JE virus
Laboratory study ····· Mitamura et al. (1939)
Larves of the Anopheles sinensis ····· Wu et al. (1940)
(3) Animals other than mosquitoes, or ecto- or endo-parasites
(a) Latent infection of pigs, cows, birds and warm or cold-blooded animals
(b) Parasitic arthropods or parasites as possible carriers of virus infection or latent infection
(c) Bat-mosquito cycle (Experimental study by La Motte (1957))

(actually there is a minor difference in timing) visit of migratory birds at least to the area west of Kanto district in early spring. In fact, Sagiyama virus isolated in Kanto district of Japan by Scherer et al. (1957) has been verified in the Malay Peninsula by Buscher et al. (1960); also Akabane virus isolated by Oya et al. (1959) can be placed under a similar control; and futhermore, it is a matter for consideration that a fairly large group of swallows and herons already come to Japan in early spring (March) prior to the bloodsucking activity of the *Culex tritaeniorhynchus* mosquitoes, but in this case, there is a great difficulty that viremia of these birds must be maintained till the time of bloodsucking activity of mosquitoes for the spread of JE virus, and the fact and the mechanism of reactivation of latent virus in the bodies of birds must be verified.

Then (2) parasitic arthropods adhered to the migratory birds can be considered: In this theory, viremia has to be effected in abult or young birds by the biting of parasites simultaneously with the bloodsucking activity of mosquitoes in early spring. However, such a condition will be too dramatic.

The latter theory or overwintering of JE virus is as follows:

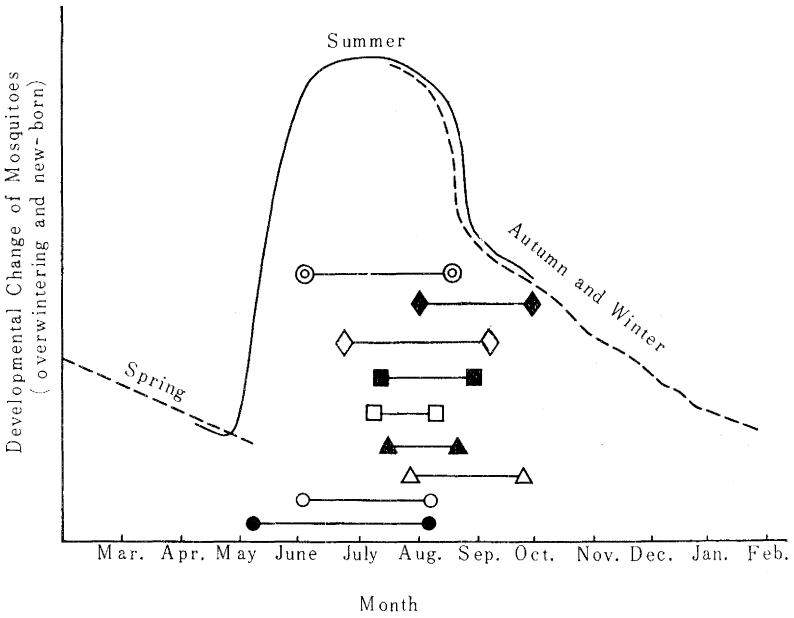
(1) Overwintering of the virus infected mosquitoes of *Culex tritaeniorhyncuhs*: The study of the *Culex tritaeniorhynchus* mosquitoes in winter has hereto been considered to be altogether difficult. However, the trial by Professor Omori et al. of the Department of Medical Zoology in authors' Institute revealed that collection of the overwintering mosquitoes of *Culex tritaeniorhynchus* at least in earlier spring is hopful (to be published in this Bulletin). Moreover, Mifune, one of the authors, enabled the overwintering of the *Culex tritaeniorhynchus* mosquitoes experi-

mentally infected with JE virus, and the transmission to susceptible animals, especially pigs, by the bite of the overwintering mosquitoes infected. The significance of the presentation by SCHERER et al. that pigs serve as an amplifier was increased by this experiment, but the problem is whether or not such virus infected mosquitoes can be verified in the natural world.

(2) The egg-passage theory is based on the laboratory verification by MITAMURA et al. (1939) and the verification of JE virus from the larves of the *Anopheles sinensis* by Wu et al. (1940). However, recent followup studies by many investigators have revealed a nagative conculsion.

(3) The role of animals other than mosquitoes such as birds, pigs, cows, and even cold-blooded animals, particularly their latent infection, and parasitic arthropods or parasites may be mentioned. In case of the latent infection, there will remain quite a difficult problem as to how the viruses in the body is reactivated to

Figure 1 Developmental Change of *Culex tritaeniorhynchus* Mosquitoes and Period of JE virus Isolation from Collected Mosquitoes



Remarks : Solid curve indicates new-born mosquitoes and dotted curve indicates overwintering mosquitoes (The portion of dotted curve for August includes anticipation).
The period of JE virus isolation from collected mosquitoes as follows :

- ●—● TAKAHASHI et al. (1964); May 19 thru August 4
- ○—○ HAYASHI et al. (1964); June 8 thru August 7
- △—△ YAMAMOTO et al. (1963); July 29 thru Septebmer 26
- ▲—▲ KITAOKA et al. (1953); July 15 thru August 20
- □—□ OYA et al. (1953); July 10 thru August 2
- ■—■ OYA et al. (1961); July 12 thru August 30
- ◇—◇ OYA et al. (1960 thru 1963); June 28 thru September 5
- ◆—◆ HURIBUT et al. (1960); August 9 thru October 6
- ⦿—⦿ WANG et al. (1962); May 31 thru August 22

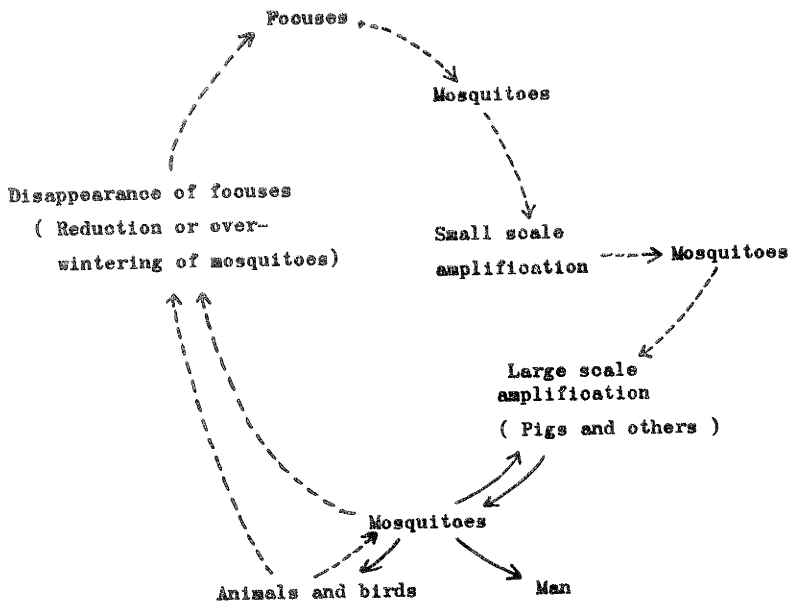
cause viremia during the period of blood-sucking activity of the *Culex tritaeniorynchus* mosquitoes. If parasitic arthropods or parasites should act the reservoir, infection must be repeated anytime and anywhere throughout the year and antibody response as the result of the infection may possibly be verified.

(4) The "mosquito—bat—mosquito" cycle which is based on the experiments by LA MOTTE (1957), may admit of consideration. However, there is an opposite phenomenon that, not only in winter, the mosquitoes of *Culex tritaeniorhynchus* is hardly seen in any cave which is an appropriate habitat of bats.

As the conculsion, neither one of the possibilities has been verified yet and the ecology of the overwintering of JE virus is still unknown. In our future research project, emphasise will be placed on the isolation of virus from overwintering mosquitoes and on the survey of JE virus infected animals in early spring and late autumn, i. e., verification of virus in the internal organs of the animals (This is to be done at first by verifying the virus antigens).

In Japan, the period that JE virus is isolated from the mosquitoes of *Culex tritaeniorhynchus* is, at earliest, May 19 (Aino Machi, Nagasaki Prefecture) and, at latest, Setember 26 (Uchihashi, Kasuya Machi, Fukuoka Prefecture). Virus isolation was made from August 22 through October 6 in the study by HURLBUT in Okinawa in 1959, and from May 31 through August 22 in the study by WANG et al. in Taiwan in 1962. In consideration of only the climatic condition, neglecting the other complicated condition, it is anticipated that virus isolation from the mosquitoes should be constantly

Figure 2 Cycle of Japanese Encephalitis Virus Infection



(Consideration on dynamics of infection according to the intensity of virulence.)

Remarks : Solid lines indicate verified portion, and dotted lines means unknown portions

available in Okinawa and Taiwan during a much longer period than at least Japan. The reality is against this anticipation. How should this reality be considered ? Not much seasonal variation is noted in the appearance of JE epidemics within the area covering at least as far as Taiwan. A tentative question may arise as to how the situation is in the area further down south, but it will return to the "carried-in" theory as previously stated.

The author has advocated the theory of "Focus in a localized situation " and, on the basis of this theory, summarized the progress of appearance and disappearance of JE epidemic as shown in Figure 2. To study the activity of JE virus in winter is no other than to study the focus itself which is shown in the Figure.

The focus of JE virus can be of any number. Originating in this focus, there will occur the primary small scale amplification which sometime later will be followed by a large scale amplification and a number of mosquitoes will be infected with JE viruses. The small scale amplification is not necessarily caused by only one kind of medium, and further contamination is developed through the medium itself. This small scale amplification takes place in early spring between the time of bloodsucking activity of the *Culex tritaeniorhynchus* mosquitoes and the time of appearance of new-born mosquitoes, and the duration is estimated to be short. It is believed that the study of JE virus infected mosquitoes or animals around the place of the small scale amplification, may lead to a clarification of the focus.

In the later part of October 1964, MIFUNE, one of the authors, experimentally infected 1043 specimens of *Culex tritaeniorhynchus* (those hatched from the larves collected in the field) with JE viruses, and tried their overwintering at three places of different circumstances. Of those, 42 specimens survived as of the last of March 1965. After having been used for experimental infection to susceptible pigs, the remaining 14 specimens further survived up to the last of April and, moreover, two out of these 14 specimens are still surving as of June 5. This period really lasted for 222 days. Among the mosquitoes that had been experimentally infected with JE virus and had overwintered, a group of 16 specimens and a group of one specimen were used respectively to bite and bloodsuck two susceptible pigs. As the result, an appearance of viremia (for 5 days) and an increase of antibody response were verified in both pigs. This fact indicated that JE virus infected mosquitoes of *Culex tritaeniorhynchus* can survive over one winter although they decrease in number and that the viruses in the mosquitoes can be transmitted to susceptible animals, and suggested that this phenomenon may possibly occur also in the natural world. Although pigs were used in this experiment as susceptible aniamls, it is anticipated that such an experiment is also applicable to some other kinds of susceptible animals. In view of this, the focus seems to be the overwintering JE virus infected mosquitoes. It is commonly known that the renewal of susceptible pigs is generally remarkable and in consideration of the habit of biting bigger animals on the part of the *Culex tritaeniorhynchus* mosquitoes (but, such uniform properties may not be the matter of argument for the mosquitoes of *Culex tritaeniorhynchus* including overwintering ones in the early stage of bloodsucking activity), pigs, as the target of the initial attack by the overwintering JE virus infected mosquitoes, are capable of partcipating in the initial spread of JE viruses. In this case, as a number of overwintering *Culex tritaeniorhynchus*

mosquitoes are seen in cow sheds or in pig sheds in the middle of April, the place of small scale amplification and that of large scale amplification seem to be identical, and accordingly it is suspected that the virus infection of mosquitoes and the increase of antibody response within susceptible animals together with the bloodsucking activity of overwintering mosquitoes should be observed in early spring. However, the reality is not so simple and there still remains a problem as to what sort of animal gave blood to the mosquitoes before they come to the cow shed. In any case, it will be quite an important verification to collect the *Culex tritaeniorhynchus* mosquitoes and isolate JE viruses from them.

We have been in vain trying to isolate JE viruses from totaled 16174 specimens in pools of 122 of the *Culex tritaeniorhynchus* mosquitoes, overwintering of which were positively identified by the Department of Medical Zoology, from among those collected with due to consideration during the period from March 1965 through the last of April 1965. The increase of antibody response within susceptible pigs has not been verified, either. The mosquitoes which commenced overwintering without bloodsucking or spawning in the preceding autumn, play no epidemiological part. If virus should be isolated from the mosquitoes (female, adult) that overwintered after bloodsucking in the preceding autumn but without bloodsucking and spawning or only with spawning (the less number of spawning, the higher rate of collection) at the time of collection, the problem will be quite simple. In contrast to this, supplementary conditions are required for the mosquitoes that have already sucked blood at the time of collection, because it is impossible to determine whether or not the animals had virus infection before blood was given to the mosquitoes. Anyway, the fact that virus isolation has been unsuccessful as stated above is deemed due to the fact that only a small number of overwintering virus infected mosquitoes have been experienced, but adeqate considerations must be given to the fact that the study of focuses other than the place of small scale amplification and of mosquitoes themselves is required. However, the study of overwintering mosquitoes of *Culex tritaeniorhynchus* must be carried out carefully and adequately, and furthermore, the study must be made on the mechanism of virus infection of new-born mosquitoes in early spring.

A great quantity of *Culex tritaeniorhynchus* mosquitoes collected in pig sheds and cow sheds are infected with JE virus in summer. After a certain period, however, the isolation of virus suddenly becomes unavailable despite the fact that collection of mosquitoes can be made. It is necessary to consider this phenomenon by distinguishing it into that during the period of activity of mosquitoes and that during the period of decrease in number of *Culex tritaeniorhynchus*. In case of the former, it is most likely that all of the susceptible animals especially pigs become to develop the antibodies, but it will be contradictory to the fact that susceptible pigs are renewed successively throughout the year. In case of the latter, it may be caused by the fact that mosquitoes hide themselves to be ready for overwintering, but there arise questions as to when they get ready for overwintering and when and how JE virus infected mosquitoes in late autumn has to be verified by verifying the overwintering JE virus infected mosquitoes prior to the offspring of new-born mosquitoes the next spring.

Summary

To study the ecology of JE virus is to investigate the focus itself. As it is suggested in Figure 2, the essential requirement for the study of the matters concerning the overwintering of JE virus infected mosquitoes consists in isolation of virus from a number of visus infected mosquitoes either in the preparatory stage or initial stage of their bloodsucking activity. At the same time, however, regardless of the kind of animal,—— Isn't it necessary to explode the current common idea that the overwintering *Culex tritaeniorhynchus* mosquitoes which are ready for, or which just started, bloodsucking activity, have a habit of biting bigger animals? ——investigation must be made on the place of small scale amplification and on the animals, other than mosquitoes, which are infected with JE virus at that place.

On the other hand, virus isolation from the *Culex tritaeniorhynchus* mosquitoes has been tried every summer in Kanto, Osaka and Nagasaki districts, but the period and efficiency of isolation quite varied from year to year. How was this variation caused? It does not seem to be caused only by the variation in number of offspring or collection of the *Culex tritaeniorhynchus* mosquitoes. Accordingly, it will be inevitably presumed that the condition of the reduction in size of focus in early autumn has an important factor. From this point of view, it is believed that the followup study of JE virus in late summer and early autumn as well as the investigation in early spring have a significant meaning in the attainment of the aim.

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日本脳炎ウイルスの越年に関する問題，林　薫，三舟求真人，七条明久，長崎大学風土病研究所病理部（主任：福見秀雄教授）

要　　約

今日，日本脳炎のウイルス学についての問題はいくつかあるが，特に流行学の上からウイルスの越年及び流行前のウイルス供給源に関する調査研究は最も困難な問題として残されている。日本脳炎の流行閑期のウイルスの消息について先人の業績を要約すると（1）ウイルスの持込（2）ウイリスの越年の2つの基本的な考え方があって，これらは更らに第1表のように種々の場合が想定されている。しかしいずれにしても自然界における確証はない。当研究所における大森等は早春の越年コガタアカイエカの採集に有効な方法を見出し昆虫学的に越年蚊と確認されたコガタアカイエカ19084個体147プールについて著者等は哺乳マウスを用いて日本脳炎ウイルスの分離を試みたが不成功に終った。これは有毒越年蚊に遭遇し得なかったからとも考えられるが，一面有毒蚊の越年以外の問題も考慮する必要があるようである。著者等の一人三舟は1964年10月下旬実験的にコガタアカイエカ1043個体に日本脳炎ウイルスを吸血感染せしめ，自然界に近い状態の3ケ所に放置し越年させ翌年（1965年）3月下旬まで42個体が生残した。このうち仔豚を供試し感染実験を行ない更らに残余の14個体は6月5日以降なお生残を続けた。抗体のない仔豚は有毒蚊1個体の刺螯吸血でウイルス血症（5日間）を来たし，また抗体上昇を認め豚のanplifier としての意義を再確認した。この事実は有毒蚊の越年を想定せしめたが，果たして自然界でこのような事実が立証されるかどうかは重要な問題である。しかし今早春，越年蚊として確認された19084個体147プールからはウイルス分離は不成功に終ったことは先述の通りである。日本脳炎の流行閑期のウイルスは早春における越年蚊及び晩秋の有毒蚊の潜行の追跡が必要であるが，一方 Focus を中心としたウイルス汚染の段階を図1のように考えるとき自然界で蚊以外の動物について調査する必要があり，それには先ず動物の日本脳炎ウイルスに対する血清抗体の有無と体内抗原の存在を検査し，その資料に基いてその動物の survivor としての役割を追究してゆくべきであると思う。

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