EXPERIMENTAL TRANSMISSION OF RUSSIAN SPRING-SUMMER ENCEPHALITIS VIRUS BY THE MOSQUITO

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Russian spring-summer encephalitis (RSSE), known as forest encephalitis or tick borne encephalitis, is disease Smorodintseff and his co-workers (1) have made clear of its epidemiological aspects, causative agent, mode of infection and preventive measures during the years 1937–39. The disease prevails mainly in the Far-Eastern Russian territory and is also reported since 1932 in European Russia and in Siberian woodlands. Kitano (2) has reported of the disease breaking out on the Manchuria-Soviet Frontiers and was successful to isolate the causative agent. Kitaoka (3) also mentioned that the disease was reported among those Japanese war prisoners engaging in wood-cutting at Sosoevka woods beyond the highlands in the north of Vladivostock in the II World War and the disease was found breaking out during the course of April 19 June 20, and about 10–16% of the total workers have contracted it, resulting in 19–50% case-mortality. Symptoms, after invasion of the virus, and as far as to the studies on the neutralizing antibody in the convalescent serum are discussed about in the report too.

The virus is spread by Ixodes persulcatus Schuize, a species of wood tick and it was also made clear by Pavlovsky (4) that Eutamia asiatica, a rodent, is known as reservoir. Casals (5) and Hammon (6) have described that causal agent is not single and there are two types, the Far Eastern and the Western types, which Kitaoka (3) has also approved by the neutralizing test with the convalescent serum from the RSSE patient and has made clear that the former type is peculiar in the nervous disturbances and extrapyramidal symptoms, while the latter chiefly presents symptoms from irritations on meninges (Yamazaki).

Smorodintseff (1) has mentioned of the disease being not spread by mosquitoes. But it is of interest to see if it may be transmitted by mosquitoes because the vector of the encephalitis occurring in summer season is, as Hammon (6) and Kitaoka (7) have mentioned, mainly a mosquito. The RSSE prevails most vigorously during late spring towards the summer when mosquitoes grow thrivingly. Well an experimental study on transmission by mosquitoes of the RSSE viruses namely, the Sophy strain (Far Eastern type) and a Western type, which were given from Dr. Hammon was carried out.

METHOD

Mosquitoes applied for the experiment were two species most commonly in Japan during summer season, namely, Culex pipiens var. pallens and C. tritaeniorhynchus, and they were collected in their larval stage in fields and hatched at the laboratory before
artificially infected with the virus by means of Mitamura's method (8), two virus strains, the Sophy Strain (Far Eastern type) and a Western type were inoculated intracerebrally respectively into mice and the brain removed therefrom were emulsified 10 per cent in a mixture of 40 per cent cane-sugar solution and defibrinated healthy rabbit-serum free from the neutralizing antibody against the virus.

The mosquito fed on a piece of absorbent cotton soaked in such emulsion and qualified if they contracted the virus by letting them bite mice of 7—10 g body-weight, as well as a test by inoculating into mice the mosquito body emulsion. The infectivity of the mosquito was expressed as LD₅₀.

Rise and fall in infectivity of the artificially infected mosquito in the course of observation was also estimated by comparing LD₅₀ of each group of the mosquito immediately after the feeding on the virus emulsion, on the 3rd, 6th, 9th, 12th, 15th and the 18th day afterwards. About 30–40 mosquitoes were emulsified which had been used for the biting experiment and picked out engorging the blood of the mice and if the number of mosquito engorging are not enough to be emulsified, the other mosquitoes in the same cage were added to make up the number and principally C. pipiens var. pallens and C. tritaeniorhynchus were mixed in half and half in number to make the emulsion.

LD₅₀ in the mosquito emulsion was worked out by intracerebrally inoculating into 3 mice each decimal dilution of Berkefeld V filtrate of the supernatant out of the emulsion of an infected mosquito suspended in 0.1 cc of 40 per cent serum broth at pH 7.4, centrifuged 4,000 r.p.m. for fifteen minutes. The virus in the mosquito emulsion immediately after the feeding on the virus emulsion is understood to have been diluted as far as to 10⁻³, for the averaged body-weight of a mosquito is about 1 mg which is

Table 1. Transmission of RSSE viruses through mosquitoes.

<table>
<thead>
<tr>
<th>No. of series</th>
<th>RSSE Far Eastern type</th>
<th>RSSE Western type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>30/Ⅶ—46</td>
<td>10/Ⅶ</td>
</tr>
<tr>
<td>Breeding temperature</td>
<td>26—30.5°C</td>
<td>28—32</td>
</tr>
<tr>
<td>Infectivity of mosquito*</td>
<td>10⁶</td>
<td>10⁶.₅</td>
</tr>
<tr>
<td>Days after engorgement</td>
<td>3 6 9 12</td>
<td>3691215</td>
</tr>
<tr>
<td>No. of mouse</td>
<td>2 2 2 2</td>
<td>2 2 2 2</td>
</tr>
<tr>
<td>No. of Sick mouse</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
</tr>
</tbody>
</table>

* Infectivity of mosquito body immediately after feeding
Fig 1. Rise and fall of virus in mosquito body after feeding (far eastern type).

usually found doubled after the feeding, and about 1 mg of the original virus suspension is diluted up to $10^{-2}$ when a mosquito is emulsified in 0.1 cc of broth. The original virus suspension is 10 per cent of infected mouse brain and the amount of virus in the mosquito body is understood as to be diluted to $10^{-3}$.

Defectiveness in inoculations of such emulsion is that the result may read negative unless more than $10^3$ of the virus is contained in mosquito body. Judgement of the encephalitis mice was confirmed in the symptoms, patho-histological findings and virus identification test, and an atmost care was paid to the effect of the breeding room temperature and moisture daily recording in the maximum and minimum.

**EXPERIMENT**

Experiments in 8 series, 4 each with the Far Eastern type and the Western type viruses have been carried out.

1. With the Far Eastern type of the virus:

The first experimental series on July 30th 1946, the second on August 10th, the third on August 23rd and the fourth on September 24th have respectively been carried out. Table 1 and Figure 1 show the results observed in the rise and fall of the virus in the mosquito body by means of inoculation with the mosquito emulsion, infectivity of the mosquito through biting experiment, number of mosquito infected and the breeding temperature and humidity then governed the experiment. The infectivity of the mosquito immediately after engorgement in each series was $10^6$, $10^5.5$, $10^5$ and $10^6$ LD$_{50}$ respectively. But it have been made clear that the virus has no longer been proved on the 3rd, 6th, 9th, 12th and 15th day afterwards in mosquito body regardless the breeding temperature at 26–33°C, 26–30°C or 21–28°C and the biting experiment also resulted in all negative on 33 mice undergone, reminding that there is no positive evidence of the fact that
Fig 2. Rise and fall of virus in mosquito body after feeding (western type).

the Sophy strain (Far Eastern type) of the RSSE is transmissible by C. pipiens var. pallens (II and IV) or by the mixture of C. tritaeniorhynchus and C. pipiens var. pallens (I and III).

2. With the Western type of the virus:

Table 1 and Figure 2 reveal the results observed in the experimental transmission of the RSSE virus Western type carried out also in 4 series on July 27th (I), August 10th (II), August 23rd (III) and September 25th (IV) respectively by using C. pipiens var. pallens and C. tritaeniorhynchus. Breeding temperature, humidity and other conditions maintained during the experiment were almost the same to those governed the experiment with the Sophy strain. Fig. 2 shows in detail of the rise and fall of the virus in the mosquito body. Amount of the virus in the mosquito body immediately after engorgement in each series was observed $10^6$, $10^6$, $10^6$ and $10^7$ respectively which made, however, a fall to $10^3$ or even lower after 3 days' time and has shown no evidence 16—18 days' later, 37 mice undergone the biting experiment with the mosquito mentioned above have failed at all in contracting the disease too.

**DISCUSSION**

There is no doubt about that RSSE is a tick borne disease, and the epidemiological factors regarding the disease also correspond with a tick borne but not with a mosquito borne disease. It is, however, a matter of interest to ascertain the fact that the disease is absolutely not related with mosquitoes because most of other encephalitis prevailing in summer season belong to a mosquito borne disease. But it has been observed that the virus, regardless the types either the Western or the Far-Eastern, have always failed in our transmitting experiment in four repetitions each during the period July to September 1946. while the positive results were obtained in the mosquito transmission experiment of both the Japanese B encephalitis and the West Nile viruses during the
same period.

Further inquisition into the negative results may perhaps make comments upon the fact that the temperature then governed over the extrinsic incubation period was rather too high in comparison with that in Siberia during the months of May and June (15—20°C). It is, however, known that there is but a very little hope to expect any increase in the amount of virus within mosquito body at the breeding temperature below 20°C, as was previously reported in the studies on Yellow Fever (Davis (9)) and Japanese B encephalitis (Mitamura (10) and Kitaoka (11)).

Experimental transmission of RSSE virus have been made by using both strains, the Far-Eastern (Sophy) type and the Western type, which were adapted to the mouse brain by brain to brain passage. Then it is questionable if these strains are suitable to transmitting experiment by mosquito. According to my experince a higher positive result is obtained by using a freshly isolated strain than by a brain to brain passed strain in the transmitting experiment of Japanese B. encephalitis by mosquitoes.

There are many kinds of the mosquito of which C. tritaeniorhynchus and C. pipiens var. pallens were used for our experiment. Well our negative result does not all deny the mosquito transmission of RSSE. Anyway the experiment should be tried with the other kinds of mosquito.

**SUMMARY**

The transmitting experiment of RSSE viruses, the Far-Eastern (Sophy) type and the Western type was undertaken with mosquitoes C. pipiens var. pallens and C. tritaeniorhynchus during the period July to September 1946. The mosquito was infected artificially with the virus emulsion and then it was put to infect a group of mice in every three days after engorgement of virus to see if the mosquitoes were capable of directly transmitting the virus to the animals, and, at the same time, the prosperity and decline of the virus maintained within such mosquito body was detected by injecting emulsion of the mosquito body into mice. The experiment was repeated four times in each strain. The virus proved within the mosquito body $10^6$ to $10^7$ LD$_{50}$ as much immediately after engorgement was observed diminishing off on and after the 3rd day, nor proved the biting experiment positive at all. In other words the experimental transmission of RSSE has resulted in negative with C. pipiens var. pallens and C. tritaeniorhynchus.

The author wishes to acknowledge the kindness of Dr Hammon in giving us the strain.

**REFERENCES**


