The Relation of the Virus Ţahyňa to Some Species of Mosquitoes of the Genera Aëdes, Culex and Anopheles

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Abstract. It was established by a laboratory test that in the mosquito species Aëdes sticticus, A. communis, A. cantans, A. excuriesis and A. flaveiscens the virus Ţahyňa can multiply and survive. An experimental transmission of virus was carried out with the species A. sticticus. The possible prolonged survival of virus in the species A. cinereus and Culex pipiens was not determined and also preliminary studies with the species A. punctor, Theobaldia annulata and the Anopheles maculipennis group were negative.

As proved by isolation tests from mosquitoes A. vexans collected in nature (BÁRDOŠ and DANIELOVÁ 1959, 1961, KOLMAN et al. 1964 and MÁLKOVA et al. 1965) and also by laboratory tests (ŠIMKOVÁ et al. 1960, DANIELOVÁ 1962a) this mosquito species participates in the circulation of the virus Ţahyňa. It is probably not the only species which is involved in the circulation of this virus in nature, although recent results suggest that it plays the main role in the transmission of the virus. The serological results show that the virus Ţahyňa is disseminated primarily in inundated and adjoining regions of bigger rivers where a mass occurrence of mosquitoes takes place every year (BÁRDOŠ 1960a, b). In the present study we have, therefore, focussed our attention to the mosquitoes occurring in large numbers in these regions, i.e. the mosquito species of the genus Aëdes. At the same time we also wanted to establish the relation of most common representatives of other genera of mosquitoes to the virus Ţahyňa.

We solved the problem in a laboratory experiment as it is often very difficult to obtain a certain mosquito species from nature in sufficient numbers for isolation test purposes, although the species may be quite numerous. The paper refers to the preliminary communication (DANIELOVÁ 1962b).

MATERIAL AND METHODS

Mosquitoes were collected both in their larval stages from which imagoes were reared in the laboratory and in their adult stages in nature. Various species were collected in a number of localities which are mentioned with respective species.
In most experiments the extraneural strain of the virus TaHyña "236" (Bádroš and Danielová 1959) was used in 7th—13th passage. The strain "181" (Kolman et al. 1964) was used exceptionally only. A special mention is made in this paper whenever this strain was used.

In most experiments the mosquitoes were infected by method of feeding on infectious tampons. Only in the experiment with the mosquito A. sticticus a viremic rabbit was used as a source of virus. The infectious suspension was prepared by mixing the viremic blood of hamsters with dilution of glucose. The ratio was adjusted according to the required titre of the infectious suspension, so that the resulting glucose concentration in the infectious suspension was 5%. The tampons were exposed to the mosquitoes for 3 hours and as was observed during this period the amount of virus in the tampon practically remained the same. The titre of the suspension was determined by titration immediately after exposure. The amount of virus ingested by mosquitoes was determined by titrating 5—6 mosquitoes immediately after ingestion. Titration was performed by diluting the suspension from mosquitoes or infectious suspension in buffered saline solution with 10% guinea pig serum and by inoculating 0.03 ml of each 10-fold dilution intracerebrally to 8—10 g white mice. The titre was calculated by the method of Reed—Muench (1938). The titre of virus in the body of mosquito is expressed by total quantity of virus in the body of one mosquito (Chamberlain, Corbistian and Siers 1954).

The persistence of virus in mosquitoes was proved by isolation test from mosquitoes at least 8 days or at most 10—14 days after the infective meal. During this period the passive survival of virus in the food ingested by the mosquito is impossible. The mosquitoes (the exact number is stated with respective species) were triturated by a cooled glass rod in a cooled glass tube and suspended in 1 ml saline solution containing 10% guinea pig serum. The suspension was centrifuged for 5 min. at 2,000—3,000 rev. The supernatant was inoculated immediately to 2—3 day old suckling mice in dose of 0.01 ml intracerebrally and 0.03 ml subcutaneously, or to 5—6 young 8—10 g mice in doses of 0.03 ml intracerebrally. In case that the young mice survived an isolation test was performed in suckling mice using the suspension stored at —70 °C. The dead mice were bacteriologically examined and the specificity of death rate was confirmed by passage or neutralization test.

RESULTS

The possible prolonged survival of virus was determined in the following mosquito species: A. sticticus, A. cantans, A. exerucians, A. flavescens, A. communis, A. cinereus, Culex pipiens, A. punctor, the group Anopheles maculipennis and Theobaldia annulata; the last three species were investigated on trial only.

Aedes sticticus(Meigen, 1838). The mosquitoes were reared in laboratory from larvae collected in the vicinity of Bratislava and allowed to feed on tampon with virus titre 3.37 log LD₅₀. The presence of the virus TaHyña was detected in the suspension prepared from 18 mosquitoes on 8th day after infection.

The persistence of the virus TaHyña in mosquitoes A. sticticus was confirmed by repeated experiment. The imagoes collected in nature became infected when feeding on tampon with a virus titre 3.64 log LD₅₀. The average amount of virus ingested by one mosquito was 1.24 log LD₅₀. After 12 days the virus was detected in the suspension from 6 mosquitoes.

In our next experiment a group of adult mosquitoes collected in nature became infected by feeding on viremic rabbit with virus titre 3.0 log LD₅₀. On the 9th day after feeding the virus was detected in the suspension prepared from 3 mosquitoes.
We tried to find out whether this mosquito species is also capable of transmitting the virus Tabyňa. The mosquitoes became infected when sucking viremic blood from tampon with a virus titre 5.3 log LD₅₀. 15 and 16 days later healthy suckling mice were exposed to the mosquitoes. 3 sucking mice which were bitten by the mosquitoes or on which the mosquitoes fed, fell ill and died and the virus Tabyňa was recovered from them.

Table 1. Survival of the virus Tabyňa in some species of mosquitoes

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Virus titre in source of infection*</th>
<th>Days of incubation period</th>
<th>Virus detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. sticticus</td>
<td>3.37</td>
<td>8.26</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3.64</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>A. communis</td>
<td>2.36</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>A. cantans</td>
<td>5.30</td>
<td>16</td>
<td>+</td>
</tr>
<tr>
<td>A. excrucians</td>
<td>2.36</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>A. flavescens</td>
<td>5.30</td>
<td>16</td>
<td>+</td>
</tr>
<tr>
<td>A. cinereus</td>
<td>2.36</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>1, 2, 3, 5, 8, 14, 23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>A. punctor</td>
<td>3.37</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>C. pipiens</td>
<td>3.16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>14, 21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.53</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.50</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>An. maculipennis</td>
<td>3.64</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Th. annulato</td>
<td>4.00</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.64</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

*) log LD₅₀.

In contrast with these laboratory results the repeated isolation tests from mosquitoes Aëdes sticticus collected in nature were negative. So far a total of 12,600 specimens were used in isolation tests from mosquitoes of this species. We therefore tried to elucidate the question as to whether the negative results are caused by a low infection rate in this mosquito species. The method employed in this test was similar to tests with the species Aëdes vexans and is described in detail in DANILOVÁ’s paper (1966). The mosquitoes reared from larvae became infected when feeding on the infective blood containing the mouse brain suspension with the strain “181” from tampon with a virus titre 5.16 log LD₅₀. After 14 days we examined individually 25 mosquitoes and the virus was recovered only from
13 of them. The infection rate was 52%, i.e. a value, by which the negative results of isolation tests from mosquitoes collected in nature (BÁRDOŠ and DANIELOVÁ 1959, 1961) cannot be explained.

*Aëdes communis* (De Geer, 1776). The mosquitoes were reared from larvae collected in the vicinity of Blatná and Dobříš. They became infected when feeding on tampon with a virus titre $2.36\log LD_{50}$. 10 days after feeding the virus was detected in the suspension prepared from 3 mosquitoes.

*Aëdes cantans* (Meigen, 1818). The imagoes collected in South Moravia became infected by sucking the viremic blood from tampon with virus titre $2.36\log LD_{50}$. On 16th day after feeding the virus was detected in the suspension prepared from 3 mosquitoes.

In the repeated experiment the mosquitoes became infected by feeding on tampon with virus titre $2.36\log LD_{50}$. The average amount of virus ingested by one mosquito was $1.50\log LD_{50}$. After 11 days incubation period the virus was detected in the suspension prepared from 21 mosquitoes.

*Aëdes excrucians* (Walker, 1856). The imagoes were collected in South Moravia. They became infected by sucking viremic blood from tampon with virus titre $2.36\log LD_{50}$. On the 16th day after feeding the virus was detected in the suspension prepared from a single mosquito.

In repeated experiment the mosquitoes became infected when feeding on infectious tampon with virus titre $2.36\log LD_{50}$. The virus was detected in the suspension prepared from 5 mosquitoes on the 10th day after the infective meal.

*Aëdes flavescens* (Müller, 1764). The imagoes were collected in South Moravia and became infected by feeding on infectious tampon with virus titre $2.36\log LD_{50}$. 2 mosquitoes were tested for presence of virus 10 days after feeding and the virus was found in them.

*Aëdes cinereus* Meigen, 1818. Larvae were collected in the vicinity of Lanžhot. The mosquitoes fed on infectious tampon with virus titre $1.5\log LD_{50}$ and the presence of virus was detected at various intervals after the infective meal in 5 mosquitoes each time. Immediately after feeding only traces of virus were recorded, but on 1st, 2nd, 3rd, 5th, 8th and 23rd day after feeding the virus was never recovered.

In an experiment, when the mosquitoes fed on infectious tampon with virus titre $3.6\log LD_{50}$ the survival of virus was not determined in them after incubation period of 10 days either.

*Aëdes punctor* Kirby, 1837. The relation of this species to the virus Šabyňa was studied in a limited number of mosquitoes which ingested the virus from infectious
tampon with titre 3.37 log LD_{50} of the "181" virus strain. 13 days after feeding a negative isolation test was performed from 3 mosquitoes.

*Culex pipiens* Linné, 1758. This species hibernates in the stage of imago and was used in several experiments with regard to its possible role in virus hibernation, but all tests proved to be negative.

The mosquitoes ingested the virus from tampon with virus titre 3.16 log LD_{50}. After the incubation period of 10 days the virus was not detected in two mosquitoes which survived. In another experiment the mosquitoes were reared from larvae collected in Prague. They ingested the virus from tampon with virus titre 2.0 log LD_{50}. In the suspension prepared from 25 mosquitoes the virus was not detected after 14 days.

When the mosquitoes ingested the virus from tampon with virus titre 1.0 log LD_{50}, it was detected in 6 mosquitoes 15 days after the infective meal.

The mosquitoes also ingested the virus from tampon with virus titre 3.53 log LD_{50} and the isolation test performed from 22 mosquitoes 14 days after feeding was negative.

The mosquitoes which were reared from larvae collected in Hradec Králové fed on infectious tampon with virus titre 4.5 log LD_{50}. 14 days after feeding the virus was not detected in 12 mosquitoes.

*Anopheles maculipennis* group. The imagoes collected in South Slovakia imbibed the virus from infectious tampon with virus titre 3.64 log LD_{50}. The average amount of virus ingested by one mosquito was 2.11 log LD_{50}. After 8 days, however, the virus was not detected in the suspension prepared from 3 mosquitoes.

The mosquitoes collected in the stage of imagoes in South Moravia imbibed the virus from tampons with virus titre 4.0 log LD_{50}. 14 days after feeding the virus Tahyňa was not detected in the suspension prepared from 6 mosquitoes.

*Theobaldia annulata* (Schrank, 1776). The preliminary study on the persistence of the virus Tahyňa in this species was negative. The virus was not detected in the body of mosquito 10 days after it was ingested from tampon with virus titre 3.64 log LD_{50}.

**Discussion**

It was established that the virus Tahyňa can survive and multiply in mosquitoes *Aedes sticticus*, *A. communis*, *A. cantans*, *A. excrucians* and *A. flavescens*, because during the incubation period used in the tests, the virus cannot only passively survive, but must multiply (Šimková et al. 1960, Danielová 1962).

The role played by the species (which are mentioned in this paper and in which the virus Tahyňa can multiply) in the circulation of virus in nature was confirmed in the species *Aedes cantans*, from which 2 strains of the virus Tahyňa were isolated in South Moravia (Máloková et al. 1965). On the other hand, isolation tests from
mosquitoes *Aedes sticticus* collected in nature (Bárdos and Danilova 1959, 1961) show that this species plays a minimal role or none whatsoever in the circulation of the virus. Considering its large numbers in nature, mainly in inundated regions, this fact seems to be very significant and interesting. Laboratory results have shown that the infection rate in this species is relatively high, so that the cause of this fact must be seen in the bionomy of this species and of the virus reservoir, which apparently excludes the possible feeding of the vector on viremic reservoir animal. The role of other mosquito species in nature must be confirmed by further isolation tests from mosquitoes collected in nature.

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**REFERENCES**


