TWO MORE MOSQUITO SPECIES PROVED AS VECTORS OF TAHYŇA VIRUS IN CZECHOSLOVAKIA

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Abstract. Two strains of TAHYŇA virus were isolated during mosquito virological testing in summer 1975 in the littoral zone of large fishponds in South Moravia. The first of them was isolated from Culex modestus mosquito, the other one from Aedes sticticus. Virus isolation from these species proved them to be vectors of TAHYŇA virus, as it was found already experimentally that TAHYŇA virus multiplies in these mosquitoes. This virus was isolated from the two species for the first time in Central Europe and from Aedes sticticus for the first time in general.

As vectors of TAHYŇA virus the following mosquito species have been ascertained so far in Central Europe: Aedes vexans, Aedes cantans, Aedes caspius, and Culiseta annulata, to which moreover we ascribe a special role in virus hibernation (Danielová and Minář 1969). Recently the virus was also isolated from Aedes cinereus mosquito (Málková et al. 1974). Besides that, additional species used in laboratory experiments are known for the fact that the virus multiplies in them (Danielová 1966), but they have not been proved as vectors in nature as yet. The isolation of TAHYŇA virus in the summer season 1975, from two of those species is described in this paper.

MATERIALS AND METHODS

Mosquitoes were collected during day attack on man in the biotope of the littoral zone of large fishponds. The collections were carried out in South Moravia at the fishponds Mýnšký, Hlohovecký, Nevyt and Starý in the vicinity of the villages Lednice and Sedlec and at dead channels of the river Dyje near the village Nejdek.

Mosquitoes were anesthetized by CO₂, identified and then immediately or after 2 – 3 days storage on dry ice virologically tested according to species and localities. From approximately 100 mosquitoes suspension was prepared by grinding in 1.5 ml of 10 % inactivated calf serum in PBS (phosphate

| Table 1. Survey of mosquito species in the littoral zone of fishponds in South Moravia and results of their virological investigation |
| --- | --- | --- |
| Mosquito species | Number of mosquitoes | Occurrence in % | Isolation tests |
| | | | total | positive |
| C. modestus | 6.771 | 89.18 | 74 | 1 |
| A. vexans | 669 | 8.70 | 8 | 0 |
| A. cinereus | 72 | 0.94 | 1 | 0 |
| A. sticticus | 53 | 0.70 | 1 | 1 |
| C. pipiens | 37 | 0.48 | 2 | 0 |
| Total | 7.592 | 100.00 | 86 | 2 |
buffered saline) with 1,000 units of penicillin and 1,000 units of streptomycin/ml. After about 1 hour’s
ice bath the suspension was centrifuged at 2,800 rev/min for 10 minutes and the supernatant was
inoculated on 1 litter of 2-3 day-old suckling mice in an amount of 0.01 ml ier and 0.03 ml scnt
per mouse. The mice were then observed for 3 weeks for symptoms of illness.
Isolated strains were identified by plaque reduction neutralization tests on WHO panels in PS
C14 cells according to De Madrid and Porterfield (1969). 100 PFU dose of virus and serially
twice diluted mouse hyperimmune serum anti-Tahyun virus (strain 181) and mouse negative serum
were used. Identification was verified by means of ier neutralization test in young 8-10 g SPF
white mice inoculated with the mixture of hyperimmune anti-Tahyun virus serum diluted 1:2 and
ten times serially diluted virus. Negative mouse serum was used as control.

RESULTS

More than 7,590 mosquitoes collected between 5–15 August 1975 were tested in
86 pools. Five species were found among them: Culex modestus, Aedes vexans, A.
cinereus, A. sticticus, and C. pipiens, the first mentioned species being the most
frequent. The species proportion and results of virological testing are given in Table 1.
Two strains of virus were isolated from these mosquitoes, which were subsequently
identified as Tahyun virus. In plaque reduction neutralization tests 100 PFU of both
strains (the 2nd passage of the strain 7,450 and the 3rd one of the strain 7,643) were
neutralized by the Tahyun antisera diluted up to 1:160; in neutralization tests
neutralization indices 4.21 log LD_{50} of virus were ascertained for the strain 7,590
and > 4.75 log LD_{50} of virus for the strain 7,643. The strain 7,590 was isolated from
100 specimens Culex modestus mosquitoes collected at the fishpond Stary; the strain
7,643 was isolated from 53 specimens of Aedes sticticus mosquitoes collected from localities
Mlynsky and Hlohovecky fishponds and the Dyje dead channels near Nejdek
and processed together. A small amount of mosquito suspension was the reason why
reisolation could not be carried out. The Tahyun virus was isolated from both species
for the first time in Czechoslovakia and from the species Aedes sticticus for the first
time in general.

DISCUSSION

The susceptibility of the two mentioned mosquito species to Tahyun virus was
already demonstrated earlier by laboratory experiments (Danielová 1966, 1968 —
unpublished results). The French authors Chippaux et al. (1970) even succeeded
to isolate the Tahyun virus from hibernating mosquitoes C. modestus. However,
we were not successful in isolating the Tahyun virus from these mosquitoes earlier,
though we worked in these localities and biotopes in previous years and tested virologically
about 47,000 of C. modestus species (Danielová et al. 1966; Málková et al.
1974). These species do not seem to play a role of a significant amplifier of Tahyun
virus, they are included into virus circulation occasionally and above all they can
replace the dominant vector in those sites, where it is absent. The proof of C. modestus
as Tahyun virus vector is important also from the viewpoint of virus hibernation, as
this species overwinters as imago. On account of analogy with the experiments of
Tahyun virus hibernation in Culiseta annulata (Danielová and Minár 1969) and
multiplication of this virus at low temperatures corresponding to those of mosquito
hibernation (Danielová 1975), we can suppose that active survival of the virus in
this mosquito species may occur. As was already mentioned above, the virus was
isolated from these mosquitoes in France during December.

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The proof of *A. sticticus* mosquito as vector of Ťahyňa virus is important with respect to the fact that it is a dominant component of calamitous mosquito population in some localities and that it might play a much more significant role there. *A. sticticus* as vector of California Encephalitis Complex viruses in nature is not known as yet and the experiment to infect it with California Encephalitis virus yielded negative results. (Reeves et Hammon 1952).

ДАЛЬНЕЙШИЕ ДВА ВИДА КОМАРОВ В РОЛИ ПЕРЕНОСЧИКОВ ВИРУСА ТЯГИНЬЯ В ЧЕХОСЛОВАКИИ

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Резюме. Во время вирусологических исследований комаров литоральной зоны больших прудов южной Моравии летом 1975 г. выделены два штамма вируса Тягина. Первый выделен от комара *Culex modestus*, второй — от *Aedes sticticus*. Так как уже экспериментально установлено, что вирус Тягина размножается в этих комарах, его выделение подтверждает их вирусоношительство. Вирус выделен от этих двух видов впервые в средней Европе, а от вида *Aedes sticticus* в первый раз вообще.

REFERENCES


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The IIIrd Symposium on Medical and Veterinary Acarocentomology was held in the building of the University in Gdańsk-Oliwa on 25—28th September 1975. It was organized by the Polish Parasitological Society and attended by specialists from Poland, Hungary, German Democratic Republic, Rumania, Yugoslavia, Federal Republic of Germany, England, Holland, USA and Czechoslovakia (Academician B. Rosický and Graduate Engineer D. Novák). Contributions in each section were included in a summarizing report read usually by the