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# TO THE SEASONAL OCCURRENCE OF THE VIRUS ŤAHYŇA

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**Abstract.** In the years 1967 and 1968 sentinel rabbits exposed to mosquitoes in the natural focus of Ťahyňa virus in Southern Moravia were tested for the presence of antibodies against Ťahyňa virus. The rabbits were exposed from the beginning of mosquito season till the second half of July in 1967 and till the second half of September next year. Antibodies against Ťahyňa virus were not found prior to the emergence of *Aedes vexans* population, although the species *Aedes cantans* which was proved as the vector of Ťahyňa virus was found in nature 7 to 8 weeks earlier. Antibodies were found in 1968 starting from July 25, i.e. approximately 4 weeks after the emergence of *Aedes vexans*.

While solving the problems of the circulation of Ťahyňa virus, besides finding vector and host ranges, it was also very important to find out the length of the active season of virus circulation, mainly its beginning. This question is important not only from the epidemiological aspect, but also for the knowledge of ecology of this virus. So far the isolation tests from mosquitoes were done in the period of largest numbers of mosquitoes, i.e. in the period considered as optimal for the virus occurrence. To make clear the question of the period of transmission we chose the method of sentinel rabbits that were exposed in nature during the whole period of mosquito activity and tested for the presence of antibodies at regular intervals. The experiment was carried out in the spring and the summer of 1967 and 1968 in Southern Moravia.

## MATERIAL AND METHODS

Experimental conditions: A lowland forest covering about 170 ha situated near the village Drnholec southwest from the town Mikulov was chosen as a study area. This forest is located on the bank of the river Dyje, which forms blind arms there and innundates a part of the forest every year thus making possible the hatching of large numbers of mosquitoes. The biotope and mosquito population occurring in this locality were described earlier by Minář (1969). A total of 21 mosquito species were found in this forest. The area was proved as natural focus of Ťahyňa virus and 22 strains of Ťahyňa virus were isolated there from 1962 to 1966 (Danielová et al. 1966, Danielová and Kolman, in litt.). The mentioned isolation experiments were carried out between the second half of July and the beginning of September.

Chinchilla rabbits weighing about 2—2.5 kg were employed as sentinel animals. They proved to be suitable for such an experiment earlier (Kolman et al. 1966). Sixteen rabbits were placed in eight cages, two per one cage. The walls were formed by parallel wire grating in 4.5 cm distance in order that the entrance of mosquitoes would be easiest. Over the top of each cage a plastic cover was placed

for protection of rabbits against rains. The cages with rabbits were fixed on stands about 1 m above the ground to be protected against predators on one side and against innundation on the other side. One cage only was placed under the roof of a feeding-rack about 2 m above the ground. The cages by twos were placed in four different sites of the forest which we considered to be different as concerns mosquito species, day activity of mosquitoes and range of mosquito hosts. Four control rabbits were placed in thermophilic oak forest with thin bush undergrowth about 30 km away from the studied locality. There are no large breeding sites in the neighbourhood. Mosquitoes are infrequent there except in the season of a maximum mass occurrence. The hair on the backs of the rabbits was shaved in order to enable the easiest mosquito feeding. The rabbits were bled by cardiac puncture every 10 days in 1967, every fortnight in 1968 respectively. Before the exposure the rabbits were tested for the presence of any antibodies.

Serological tests: Sera samples were stored at − 20 °C. Sera were tested both by the complement fixation test (CF) and by neutralization test utilizing tissue cultures. The sera were inactivated for 30 minutes at 56 °C before testing. CF test was carried out by usual method in test tubes using 16 units of antigen prepared from 10 % brain suspension purified with chloroform (Ilyenko 1954) and two units of complement. Incubation was done overnight at +4 °C.

Neutralization test was carried out in stable line of rabbit lung cells (Szantó 1960) in 1967 and in stable line of green monkey *(Cercopithecus aethiops)* kidney cells in 1968 respectively. The latter line was obtained by courtesy of Dr. Janda from the Instituto of Sera and Vaccines in Prague. The method of the neutralization test was published earlier (Danielová and Marhoul 1967).

Sera samples were tested after each collection of blood and positive sera only were tested once more in one test at the end of the whole experiment.

## RESULTS

In 1967 the rabbits were exposed in nature from April 20 till July 25. Ten species of mosquitoes were found during this period. The succession of spring mosquito species was studied in spring by regular collections of mosquitoes which were then employed for isolation experiments (Danielová et al. 1971). In April mostly overwintering species of mosquitoes were found, namely *Anopheles maculipennis* s.l., *Culiseta annulata*, *Culiseta alaskaensis*, *Anopheles claviger* and *Culex pipiens*, but they were very rare. The early spring species *Aedes communis* was more abundant than the others, but did not reach high numbers. During irregular inspections in the course of day we did not observe that the rabbits were attacked by mosquitoes. At the beginning of May *Aedes cantans* mosquito appeared and was more numerous than other species in the second half of May. At that time also *Aedes cinereus* and *Aedes flavescens* mosquitoes were found, but very infrequently. The spring population of *Aedes cantans* mosquito survived till summer. *Aedes sticticus* mosquito was rare. At the beginning of July *Aedes vexans* mosquito bred and reached much higher numbers than *Aedes cantans*. From the half of July the numbers and the activity of *Aedes vexans* mosquito were so high that the stay in the forest was extremely unpleasant because of molestation by mosquitoes. The rabbits were heavily attacked and they had scabs arround their ears and on their shaved backs. Mosquito calamity was finished by insecticide treatment on July 24. Mosquitoes were completely destroyed and thus the experiment was interrupted.

From the beginning of the experiment till July 25, 1967 no antibodies against Ťahyňa virus were found in the blood of the rabbits.

In 1968 the rabbits were exposed from April 25 to September 25. Mosquito population and succession of mosquito species were similar to those of the previous year, therefore they are not described in detail. Only the emergence of *Aedes vexans* started a little earlier, at the end of June, and its numbers were rather lower thanks to successful insecticide treatment in the previous year.

From April 25 to July 9 no antibodies against Ťahyňa virus were found. Antibodies were found later in the blood of two rabbits. Both rabbits were placed in a young thick willow and alder tree forest that was usually flooded by water, the usual mosquito breeding place where the largest number of mosquitoes occurred during the experiment.

Neutralization and complement fixing antibodies were found in the blood of the rabbits Nos. 25 and 11 starting July 24 and August 8 respectively. The blood of the latter was not tested from technical reasons on July 24. Table 1 shows that the titre of neutralizing antibodies of the rabbit No. 25 reached the highest level in the first positive testing already, so that this rabbit can be considered to have been infected at the beginning of July (Málková et al. 1969, Šimková 1963). Antibody titre courses of both positive rabbits are given in Table 1. Neutralizing and complement fixing antibodies of the rabbit No. 11 reached the highest level as early as August 8, suggesting that the rabbit infection occurred before July 24.

**Table 1.** Occurrence of antibodies in experimental rabbits in 1968

| Date of blood collection | Antibody titre | | | |
|---|---|---|---|---|
| | Rabbit No. 11 | | Rabbit No. 25 | |
| | CF | NT | CF | NT |
| 25. 4.—9. 7. | 0 | 0 | 0 | 0 |
| 24. 7. | — | — | 32 | 256 |
| 8. 8. | 128 | 128 | 64 | 128 |
| 21. 8. | — | 128 | **) | **) |
| 3. 9. | 16 | 128 | | |
| 24. 9. | 8 | 128 | | |

*) In reciprocal value.
**) Rabbit died.
— Not done.

As follows from the experiments in 1967 and 1968 in the mentioned locality the development of Ťahyňa virus circulation did not occur before the beginning of *Aedes vexans* mosquito wave that used to be here at the end of June or at the beginning of July. Exceptionally we observed the first *Aedes vexans* breeding as late as in the half of August (1966). From the epidemiological aspect the period of July and August, eventually of September, can be thus considered as important.

## DISCUSSION

Late occurrence of antibodies ascertained is rather surprising, as *A. cantans* mosquito which was proved as Ťahyňa virus vector by laboratory experiments (Danielová 1966) and by isolation tests from nature (Málková et al. 1965) occurs in the studied area from the beginning of May. Also the less abundant species *A. communis*, *A. flavescens* and *A. excrucians* which proved as susceptible to Ťahyňa virus (Danielová 1966) occurred in May. It remains to be seen whether the used method of sentinel animals is not too rough to detect single cases of virus circulation prior to mass occurrence of the dominant vector, though we consider this method to be more effective than isolation experiments from mosquitoes in spring time, when there are low numbers of mosquitoes. Considering the results published earlier (Danielová and Minář 1969, Danielová et al. 1970) that the virus survived during the nonvegetation period in *Culiseta annulata* mosquito, there would be a possibility that in transmission of the virus this mosquito species could be in direct connection with the dominant vector *Aedes vexans* without the help of spring mosquito species.

The results obtained are in accordance with the results of Aspöck, Kunz and Pretzmann (1970) who made isolation tests from moquitoes in 1967 throughout the whole

mosquito season starting from April 13. Even if the conditions in the natural focus at Lake Neusiedler are rather different and *Aedes caspius* and *A. dorsalis* mosquitoes are the dominant vectors there, the Ťahyňa virus was isolated in the second half of June after the appearance of *A. caspius* and *A. dorsalis*.

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## ПРИМЕЧАНИЯ К СЕЗОННОМУ ПОЯВЛЕНИЮ ВИРУСА ТЯГИНЯ

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**Резюме.** В 1967 и 1968 гг. проводились исследования на наличие антител против вируса Тягиня в подопытных кроликах, на которых кормились комары в природном очаге вируса Тягиня в южной Моравии. Кроликов подвергали налетам комаров с самого начала их сезона по вторую половину июля 1967 г. и по вторую половину сентября следующего года. Антитела против вируса Тягиня не находили перед появлением популяции комара *Aedes vexans*, не смотря на то, что вид *Aedes cantans*, установленный переносчик вируса Тягиня, был обнаружен в природе 7—8 недель до этого. Антитела находили в 1968 г. начиная с 25-го июля, т. е. приблизительно 4 недели после появления комара *Aedes vexans*.

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