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EXPERIMENTAL TRANSMISSION OF ŤAHYŇA VIRUS (CALIFORNIA GROUP) TO WILD RABBITS (ORYCTOLAGUS CUNICULUS) BY MOSQUITOES

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DEDICATED TO ACADEMICIAN J. KRATOCHVÍL ON THE OCCASION OF HIS 70TH BIRTHDAY

Abstract. Two wild rabbits (*Oryctolagus cuniculus*) were subcutaneously infected with a dose of 2.6 dex. i. c. LD_{50}/0.02 ml of extraneurally passaged Ťahyňa virus strain "236". During the 24—72 hours interval p. i. viremia was demonstrated in both animals ranging from 1.0 to 3.42 dex. i. c. LD_{50}/0.02. Mosquitoes *Aedes vexans*, which had fed on them, transmitted the virus to one of three other rabbits. During 24—96 hours interval after terminated feeding of infectious mosquitoes viremia was detected in this animal ranging from 0.49 to 4.08 dex. i. c. LD_{50}/0.02 ml. Seroconversion was revealed by means of plaque reduction neutralization test 11 days after infection.

While studying the ecology of arboviruses it is important to define the role of mammals in the circulation of these pathogenic agents. The results of serological studies have revealed that the wild rabbit is a host of the Ťahyňa virus (Hannoun et al. 1969) and that it takes part in the virus circulation. However, there has been no proof so far as to the extent of its participation. Viremia was described in the domesticated form of rabbit after experimental infection (Šimková et al. 1960) and after infection by mosquitoes in nature (Kolman et al. 1966).

MATERIAL AND METHODS

Experimental animals. Three males and two females of the wild rabbit, 2.5—4 months old, were trapped in the district of Břeclav (south Moravia) three weeks before the experiment was started. The rabbits were placed in special cages to prevent any access to them by mosquitoes. Before the experiment all animals were serologically examined.

Virus. Extraneurally passaged strain TAH "236" $Ma_5Oc_3Ma_1$ (Bárdoš et al. 1961) was subcutaneously inoculated into rabbits in the region of dorsal part of neck. The supposed dose of virus 2.6 dex i.c. LD_{50} in 0.5 ml of inoculum was verified by a simultaneously running i.c. titration on 1—2 day-old white mice. Blood from the ear vein of rabbit was used for virus isolation. It was i.c. inoculated into 1—2 day-old white SPF mice.

Serologic investigation. The sera were tested in a plaque reduction neutralization test using PS cells (Rödl et al. 1977).

Mosquitoes. *Aedes vexans* was collected in the inundated forest between Lednice and Nejdek localities (district of Břeclav) in mid-July, i.e. closely before the beginning of experiment. Blood-sucking was provided by attaching a box with mosquitoes to the abdomen of rabbit for 2 hours.

Technique. Two rabbits (No 1 and No 2) were s. c. inoculated with the above described dose of virus. At the intervals of 24, 48 and 72 hours after infection mosquitoes were allowed to feed on them, while viremia was simultaneously being detected. (Table 2). Fifteen days after the last feeding the mosquitoes were allowed to feed on rabbits Nos 3, 4 and 5. Mosquitoes, which had fed for 24 hours on both experimentally infected rabbits, were allowed to feed on rabbit No 3, all mosquitoes which had fed for 48 hours were allowed to feed on rabbit No 4 and mosquitoes which had fed for 72 hours, were allowed to feed on rabbit No 5. The feeding on rabbits Nos 3, 4 and 5 took place on one day and all three rabbits were tested for viremia at an interval of 24—96 hours after mosquito feeding.

RESULTS

Viremia in rabbits after s.c. infection. Viremia was detected in both infected rabbits at intervals of 24—72 hours after inoculation with a dose of 2.6 dex. i.c. LD_{50} of virus (Table 1). Rabbit No 1 revealed slightly higher values during all days of experiment, and in both rabbits the highest viremia was detected 48 hours after infection, reaching the values 3.0 and 3. 42 dex. i.c. LD_{50}/0.02 ml. Table 2 presents a survey of the number of mosquitoes which had fed on the infected rabbits, and after 15-day-interval fed again on rabbits Nos 3, 4 and 5.

Transmission of virus by infected mosquitoes. Of those animals exposed to infected mosquitoes viremia was detected only in rabbit No 4. (Table 3). The highest value of viremia 4.08 dex. i.c. LD_{50}/0.02 ml was reached on the second day. The eleventh day after infection by infected mosquitoes antibodies were detected by plaque-reduction test. Rabbits Nos 3 and 5 formed no antibodies.

Table 1. Viremia in rabbits experimentally infected with a dose of 2.6 dex i.c. LD_{50}/0.02 ml of

Rabbit	Viremia in hrs. p.i.			Antibodies in days	
	24	48	72	0	14
1	1.58*	3.42	3.19	<4**	4,096
2	1.0	3.0	2.67	<4	4,096

* dex i.c. Ld_{50}/0.02 ml, ** reciprocal titres in PRNT

Table 2. Number of engorged mosquitoes *A. vexans* on experimentally infected rabbits following a 2-hour-feeding

Rabbit No.	Hrs p.i.		
	24	48	72
1	130*	132	136
	67/43**	71/39	80/37
2	100	90	105
	57/30	65/36	88/42

* a total number of fully engorged females of *A. vexans* on particular rabbits

** numerator of fraction indicates number of mosquitoes which survived a 15-day-interval, denominator indicates number of mosquitoes which engorged to repletion again on rabbits Nos 3—5

Table 3. Viremia in rabbit No 4 infected by mosquitoes

	Hrs p.i.				Antibodies in days		
Values of viremia in dex. i.c. LD_{50}/0.02 ml	24 0.49	48 4.08	72 2.64	96 1.29	0 <4	11 512	26 4,096

DISCUSSION

If viremia in s.c. infected wild rabbits is compared with Ťahyňa viremia in domestic rabbits (Šimková 1963), it may be concluded that in both, the wild and domesticated forms, it has an approximately similar course and depends on the individual sensitivity of rabbits. Wild rabbits Nos 1 and 2 reveal similar maximum values of viremia 48 hours after infection. In the domesticated form the maximum values 3.91 dex. i.c. LD_{50}/0.02 ml of virus were detected by us as late as 72 hours after inoculation with a similar dose of virus (unpublished), in agreement with data obtained by Šimková (1963). The course of viremia in the wild rabbit infected by mosquitoes is also similar to viremia in wild rabbits inoculated s.c. Maximum values were also reached on the second day after infection. The absence of viremia in rabbit No 3 is not surprising, because the virus level in blood of Nos 1 and 2 wild rabbits 24 hours after infection was lower than the threshold value of mosquito susceptibility (Danielová 1966).

The mosquitoes which transmitted the virus to rabbit No 4, had become infected on Nos 1 and 2 rabbits 48 hours after their inoculation. Consequently, they imbibed the blood with a maximum value of viremia (3.42 and 3.0 dex. i.c. LD_{50}/0.02 ml). The values of viremia in Nos 1 and 2 rabbits 72 hours after infection should have been sufficient to infect the mosquitoes, but the absence of antibodies in the blood of rabbit No 5 shows that the latter has not been infected. In view of the fact that we investigated summer dynamics of the entire mosquito synusium, we can assert that *A. vexans* specimens used in our experiment belonged to the first brood of the current year. The probability of their being naturally infected is therefore minimal.

The role of the domestic rabbit in the ecology of the Ťahyňa virus was experimentally demonstrated (Šimková et al. 1960, Kolman et al. 1966, Aspöck et al. 1971, Danielová 1972). The evidence that the wild rabbit reacts to this virus similarly, as far as the course of viremia and antibody formation are concerned, and that it produces a sufficient viremia after being infected by biological vector, supports our assertion that this animal plays a role in the circulation of Ťahyňa virus in nature. According to classification of Bárdoš and Rosický (1979) the wild rabbit may be designated as very probable host and very probable host-amplifier. Due to its considerable ecological valency it often inhabits biotopes where it encounters *A. vexans* mosquitoes. It has a great reproductive potency, the young may be found during the whole vegetation period until autumn. In favourable years it can kindle as many as five times, each litter consisting of 10 young. The home range of this species is not wide, the individuals often stay in the vicinity of burrows, so that the population density may be quite high (as many as several scores per 1 ha).

On the basis of the presented data and on the numbers of the wild rabbit in nature it may be assumed that this species is an important link in the natural circulation of the virus.

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ЭКСПЕРИМЕНТАЛЬНАЯ ПЕРЕДАЧА ВИРУСА ТЯГИНЯ (CALIFORNIA GROUP) ДИКИМ КРОЛИКАМ (*ORYCTOLAGUS CUNICULUS*) КОМАРАМИ

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Резюме. Двух диких кроликов (*Oryctolagus cuniculus*) подкожно заразили экспериментально пассажированным штамом „236" вируса Тягиня в дозе 2.6 dex. i. c. LD_{50}. В течение 24—72 часов после заражения у обоих животных обнаружена вирусемия в пределах от 1.0 до 3.42 dex. i. c. LD_{50}/0.02 мл. Комарами *Aedes vexans*, которые кормились на этих кроликах, вирус передан одному из следующих трех кроликов. В течение 24—96 часов после окончания кормления инфицированных комаров у этого кролика обнаружена вирусемия в пределах от 0.49 до 4.08 dex. i. c. LD_{50}/0.02 мл. Сероконверсия установлена при помощи реакции нейтрализации по редукции бляшек 11 дней после заражения.

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