Acta virol. 13 : 103–107, 1969

BEHAVIOUR OF TICK-BORNE ENCEPHALITIS VIRUS IN ARTIFICIALLY INFECTED *IXODES PERSULCATUS* TICKS

Z. N. KONDRASHOVA, A. N. ALEXEYEV

Research Institute of Viral Infections, Sverdlovsk; and Central Research Institute of Disinfection, Ministry of Public Health of the Russian S.F.S.R., Moscow, U.S.S.R.

Received November 12, 1968

Summary. — The behaviour of tick-borne encephalitis (TE) virus in *Ixodes persulcatus* ticks was studied by the method of forced dosed feeding. The infectivity and the virus titre in ticks depended 1) on the dose of inoculated virus and 2) on the time of virus penetration into the ticks, that is at the beginning or the end of the feeding period. In the former case there occurred 100% infection of ticks along with a rise of virus titre as compared with the initial level, and in the latter case the virus was gradually eliminated from the ticks.

Introduction

Most studies on the relationship of TE virus to the vector were performed by the method of feeding the ticks on the animal-donor. This method, however, does not allow to evaluate the quantity of virus ingested by the tick (Shubladze and Serdyukova, 1939; Pavlovsky and Solovyov, 1940, 1941; Chumakov, 1944; Chumakov *et al.*, 1945; Dumina, 1958*a*, *b*, 1960; Gorozhankina, 1965; Benda, 1958*a*, *b*; Řeháček, 1960, 1962, 1965, etc.). Only in some experiments the authors used parenteral inoculation of the virus directly into the body of the tick *Ixodes ricinus* (Řeháček, 1965, 1966). Apart from some technical difficulties this method of inoculation allows the virus to bypass the "gut barrier" of the tick (Řeháček, 1965), which possesses certain defensive functions.

The above-mentioned difficulties have been overcome by the method of artificial forced dosed feeding (and infecting) of insects, developed by one of us (Alexeyev, 1965) and adapted for infecting ticks. By this method, female *Ixodes persulcatus* ticks were infected with given doses of TE virus. The results of this investigation are presented below.

Materials and Methods

Ixodes persulcatus ticks, collected in a focus of TE, were used. The ticks were infected from a capillary tube of a device for forced dosed feeding of insects (Alexeyev, 1965), either before feeding them on white mice ("pre-feeding"), or after a 5-day period of feeding on mice ("post-feeding"). The ticks were fed with a 25% suspension of brains from mice infected with the Sofin strain of TE virus. The suspensions were prepared by homogenizing the brains with fresh haemolyzed mouse blood and centrifuged at 13,000 rev/min at 4° C for 10 minutes. The titre of virus was determined in each suspension lot. To determine the titre of virus in ticks, each of them was weighed and homogenized in physiological saline with added antibiotics to make a 10% suspen-

sion. In the same way 10% suspensions from the half of the laid eggs were prepared. The larvae were pooled in groups of 100 each and ground in 0.5 ml of physiological saline. The virus was titrated in 4—5 g infant mice and the LD_{50}/0.03 ml values were calculated by the method of Reed and Muench. The amount of virus ingested by one tick, expressed in LD_{50}, was estimated from the volume of the liquid ingested by the tick and its virus titre.

"Pre-fed" ticks ingested on the average 0.52 μl and "post-fed" ticks 4.0 μl.

The term "sterile" tick is used to denote ticks spontaneously not infected with the virus.

Results

The ticks used in the experiments were collected in nature. The rate of spontaneous infectivity of female ticks, when fed individually on white mice under a patch, amounted to 19.5% (46 females were examined). This figure

Table 1. Changes in TE virus titres Ixodes persulcatus ticks after "pre-feeding" with virus suspension and later feeding on white mice

Titres of virus suspension used for feeding (log LD_{50}/0.03 ml)	No. artificially infected ticks	No. of ticks with the given virus titre (log LD_{50}/0.03 ml)			
		1.0—1.9	2.0—2.9	3.0—3.9	4.0—4.9
1.0—1.5	2	—	2	—	—
2.0—2.9	10	2	—	3	5
3.0—3.9	2	—	2	—	—
4.0—4.9	5	3	1	1	—
Total No. of ticks	19	5	5	4	5

is in agreement with the average infectivity of ticks in the given focus of infection (10.2% according to the average for a number of years). The titre of virus in spontaneously infected ticks did not exceed $10^{2 \cdot 4}$ LD_{50}. When "pre-feeding" females with virus suspension, all of them became infective. This was determined by titration of virus both in the ticks themselves and in the brains of mice-donors. The titre of virus in infected ticks was in most cases higher than the initial one, as can be seen from Table 1. The highest titre was found in ticks infected with suspensions having a titre of 2.0 to 2.9 log LD_{50}, that is with doses which are not maximal, but rather moderate. Similar results were obtained by one of us while infecting mosquitoes with leptomonads, and fleas with plague microbes (Safyanova and Alexeyev, 1967*a*, *b*).

After "pre-feeding" non-sterile ticks, they became 100% infected and the number of cases of virus transission by bite to the mouse on which they were feeding equally increased. Spontaneously infected ticks, that had not been "pre-fed" with virus, transmitted the virus by bite in 9 out of 46 cases; the "pre-fed" ticks did so in 13 out of 18 cases. The virus titre in such ticks was from 1.5 to 2.5 log units higher than in spontaneously infected females.

Two such ticks laid eggs. One female "pre-fed" with a suspension, the virus titre of which was 10^2 LD_{50}, contained more than 10^4 LD_{50} of virus

Table 2. The behaviour of TE virus in Ixodes persulcatus ticks "post-fed" with virus suspension

Titre of virus suspension used for feeding (log LD_{50}/0.03 ml)	No. of infected ticks	No. of ticks with the given virus titre (log LD_{50}/0.03 ml)			
		Virus not detected	1.0—1.9	2.0—2.9	3.0—3.9
2.0—2.9	3	2	1	—	—
3.0—3.9	8	1	5	2	—
4.0—4.9	10	7	1	2	—
5.0—5.9	4	—	1	2	2
Total No. of ticks	25	10	8	5	2

and it infected the mouse by bite. Half of the eggs laid by this tick was assayed for virus and a titre of $10^{1.48}$ LD_{50} was found. In 5 pools of larvae that had hatched from the other half of eggs, the virus titres varied from $10^{1.0}$—$10^{2.72}$ LD_{50}. In another case the tick did not infect the mouse by bite and it is possible that it had not been spontaneously infected. This tick was "pre-fed" with a suspension the titre of which was 10^4 LD_{50}; at oviposition, it contained virus in a titre of 10^3 LD_{50}. In half of the laid eggs, the virus titre was higher than 10^4 LD_{50}. In five pools of larvae that had hatched from the other half of eggs the virus titres varied from $10^{1.66}$ to $10^{3.0}$ LD_{50}.

Table 3. Elimination of TE virus from "post-fed" female ticks at different intervals after infection

Ticks	Intervals from infection to titration in days			Total number of ticks
	5	10—15	20—25	
Tested	5	8	12	25
Containing virus	4 (80%)	6 (75%)	4 (33%)	14 (56%)

In the second series of experiments, female ticks which had almost fully engorged on mice for 5 days were taken off and forcibly "post-fed". The ticks fed on infected mice were excluded so that these experiments may be considered as if carried out on sterile ticks. Titration of virus in such ticks was performed at different intervals after "post-feeding" with virus.

As seen from Table 2, the virus titre in these ticks never exceeded that in the suspension used for infection of the ticks. Moreover, active excretion of virus into the environment when feeding the tick from the capillary was noticed in all three cases investigated. Virus was isolated from the faeces in the first passage. As shown in Table 2, the greatest number of ticks (10) completely eliminated the virus; the longer the interval between "post-feeding" and titration, the greater the number of sterile ticks (Table 3).

It should be kept in mind that at the moment of feeding all of the females were infected, and that at the time of oviposition (20—25 days later) about

half of them appeared to be not infected. Nevertheless, in the females in which the virus was found, it could multiply to an amount sufficient for infecting the generative organs. Thus, one "post-fed" female tick laid eggs which contained the virus in a titre higher than 10^4 LD_{50}.

Discussion

First, attention should be drawn to the fact that infectivity of ticks depends on the time of virus pentration into them. To our knowledge, this fact has not yet been reported. The ticks, although given very small doses of virus in micro-volumes of suspension (on the average 0.52 μl), nevertheless, were all infected and contained virus in rather high titres. On the other hand, in half of the ticks "post-fed" with virus suspension after feeding on mice, the virus could not establish itself and its titres, regardless of the great volume of ingested suspension (up to 14 μl, on the average 4 μl), did not reach the initial infecting level.

When the ticks were "pre-fed" before feeding on mice, the best results were reached with moderate infecting doses. When "post-fed", the titre of virus in ticks was the higher the greater was the infecting dose. In the latter case the virus in the ticks behaved in the same manner as in the experiments of Řeháček (1966) who observed that virus reproduction was the fastest in ticks infected with the highest dose. Thus we may conclude that TE virus reproduction in ticks depends not only on the method of virus inoculation and the infecting dose, as Dumina (1958*a*, *b*, 1960) believes, but also upon the physiological state of the vector.

Balashov (1966) stated that at the beginning of feeding the reproduction of prohaemocytes and connective tissue cells and the activity of hypodermic cells is resumed, whereas at the end of feeding all these processes fade away. Apparently, incorporation and reproduction of TE virus in ticks takes place when it penetrates into the tissues during their active stage of development and the reproduction of their cell elements. This is in agreement with the observations of Řeháček (1966) who noted that, when the virus was inoculated parenterally, it was mostly found in significant amounts in the hypodermis and haemolymph cells. Evidently, the greater reproduction of virus in the tissues of generative organs could be explained on the same basis.

References

Alekseyev, A. N. (1965): Forced dosed feeding of insects (in Russian). *Med. Parazitol.* **4**, 467—471.

Balashov, Yu. S. (1966): Morphological and ecologo-physiological peculiarities of ticks of the superfamily Ixodidae — parasites and vectors of infections (in Russian). DSc thesis, Leningrad.

Benda, R. (1958*a*): The common tick "*Ixodes ricinus* L." as a reservoir and vector of tick-borne encephalitis. I. Survival of the virus (strain B 3) during the development of the tick under laboratory conditions. *J. Hyg. Epidem.* (*Praha*) **2**, 314.

Benda, R. (1958*b*): The common tick "*Ixodes ricinus* L." as a reservoir and vector of tick-borne encephalitis. II. Experimental transmission of encephalitis to laboratory animals by ticks at various stages of development. *J. Hyg. Epidem.* (*Praha*) **2**, 331.

Chumakov, M. P. (1944): Study on ultraviral encephalitides. VI. Transmission of tick-borne encephalitis virus to the progeny in ixodid ticks and the problem of natural reservoirs of this infection (in Russian). *Med. Parazitol.* **13** (**6**), 38—42.

Chumakov, M. P., Petrova, S. V., and Sondak, V. A. (1945): Study on ultraviral encephalitides. VII. Artificial adaptation of tick-borne encephalitis and Japanese encephalitis viruses to different tick species of the family Ixodidae. *Med. Parazitol.* **14** (1), 18—23.

Dumina, A. L. (1958*a*): Experimental study on the infestation of the tick *Ixodes persulcatus* by tick-borne encephalitis virus when sucking blood from immune animals (in Russian). *Vop. Virus.* **3**, 156—158.

Dumina A. L. (1958*b*): Experimental data on the infestation of the *Ixodes persulcatus* tick. with tick-borne encephalitis virus (in Russian), p. 46 — 47. In Virusnye neiroinfektsii, Moscow.

Dumina, A. L. (1960): Experimental data on the infestation of *Ixodes persulcatus* ticks by tick-borne encephalitis virus (in Russian). *Vop. med. Virus.* **6**, 132.

Gorozhankina, T. S. (1965): Data on the transovarial transmission of tick-borne encephalitis virus by Ixodid ticks. Abstract of thesis, Leningrad.

Pavlovsky, E. N., and Solovyov, V. D. (1940): Experimental investigations on the circulation of tick-borne encephalitis virus in the organism of the tick vector (*Ixodes persulcatus*) (in Russian). *Archiv biolog. Nauk* **59** (1—2), 111—113.

Pavlovsky, E. N., and Solovyov, V. D. (1941): On the circulation of spring-summer encephalitis virus in the organism of the tick vector *Haemaphysalis concinna* Coch. (in Russian). *Tr. VMA im. S. M. Kirova* **25**, 9—18.

Řeháček, J. (1960): Experimental hibernation of the tick-borne encephalitis virus in engorged larvae of the tick *Ixodes ricinus* L. *Acta virol.* **4**, 106—109.

Řeháček, J. (1962): Transovarial transmission of tick-borne encephalitis virus by ticks. *Acta virol.* **6**, 220—226.

Řeháček, J. (1965): Development of animal viruses and rickettsiae in ticks and mites. *Ann. Revs. Entomol.* **10**, 1—24.

Řeháček, J. (1966): Cultivation of tick-borne encephalitis virus in parenterally inoculated ticks. *Acta virol.* **10**, 230—235.

Safyanova, V. M., and Alekseyev, A. N. (1967*a*): Experiments on the susceptibility of mosquitoes to various strains of leptomonads (in Russian). Parazitologiya **1**, 191—199.

Safyanova, V. M., and Alekseyev, A. M. (1967*b*): Susceptibility of *Phlebotomus papatasis* and *Serjentomia arbaklensis* Perf. to leptomonads of different serological groups in the experiment (in Russian). *Med. Parazitol.* **5**, 580—586.

Shubladze, A. K. and Serdyukova, G. V. (1939): *Ixodes persulcatus* tick as vector of tick-borne encephalitis (in Russian). *Arch. biolog. Nauk* **56** (2), 121—131.