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EXPERIMENTAL STUDY OF THE REPRODUCTION OF KARSHI VIRUS (*TOGAVIRIDAE, FLAVIVIRUS*) IN SOME SPECIES OF MOSQUITOES AND TICKS

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Summary. — The strain Kaz-816 of Karshi virus was isolated in 1976 from *H. asiaticum* ticks collected in the North of Central Asia (Alma-Ata region of the Kazakh Soviet Socialist Republic). Both ticks and mosquitoes can be vectors of Karshi virus as proved experimentally by reproduction of the virus in *Hyalomma asiaticum* and *Dermacentor daghestanicus* ticks and *Culex pipiens molestus, Anopheles atroparvus* and *Aedes aegypti* mosquitoes as well as by transmission to newborn mice by the bite of infected mosquitoes.

Key words: Karshi virus; ticks; mosquitoes; virus reproduction in vectors; transmission

Introduction

The development of the vast territories of Kazakhstan, land irrigation and peapling will be associated with significant changes of the ecological situation. These changes, in their turn, will affect the epidemiological situation which will be mainly due to the changes of the number and composition of vector species and their feeders in circulation of arbovirus infections at the territory under development. Therefore, it is useful to reveal experimentally the potential arbovirus vectors. The present research deals with Karshi virus (strain Kaz-816) isolated in Kazakhstan, which is antigenically close to the West Nile (WN) virus.

The Karshi virus (*Togaviridae, Flavivirus*) was isolated from *Ornithodoros papillipes* ticks collected in the burrows of *Rhombomys opimus* in Uzbekistan in 1976 (Lvov *et al.*, 1976). The strain Kaz-816 was isolated in Kazakhstan in 1976 from *Hyalomma asiaticum* ticks collected from a camel and identified as a Karshi virus strain. Both strains appeared identical (Karimov *et al.*, 1978). As experimental vectors were chosen *D. daghestanicus* ticks and *C. p. molestus*, *An. atroparvus* and *Ae. aegypti* mosquitoes, representatives of the genera widespread throughout the studied territory, and also *H. asiaticum* ticks from which the Karshi virus was isolated in nature (Karimov *et al.*, 1978).

Materials and Methods

Virus. Karshi virus, strain Kaz 816 was isolated from *H. asiaticum* ticks (Karimov *et al.*, 1978) The 7 days incubation period of primary infection in suckling albino mice was reduced to 4—5 days in the following passages, the virus titre in the mouse brain suspension reaching 7 log LD_{50} /0.02 ml. The strain was proved pathogenic for 6—8 g albino mice after intracerebral infection. The virus was found to be sensitive to ether and sodium deoxycholate. The virus agglutinated goose red blood cells within the 6.0—7.0 pH range, optimal pH value being 6.6. In the laboratory the virus underwent 6 passages.

Ticks. Laboratory colonies of *H. asiaticum* and *D. daghestanicus* ticks were used. The ticks were infected using the equipment for dosed feeding of vectors (Alekseev, 1965, 1971). The mouthparts of the fixed tick were introduced into the capillary filled with virus-containing suspension. The other end of the capillary was inserted into the lumen of the micropipette. The vol of the consumed liquid was registered by the shift of the micropipette disk, observed on the screen of projection microscope. On the average, the tick consumed 0.1 μl of 20% virus-containing suspension of the albino mice brain mixed with defibrinated mouse blood at 1 : 1 ratio. The virus titre in the suspension was 6.0 log LD_{50}/0.02 ml. The ticks were divided into 2 groups. In the 1st group they were given the virus-containing mixture only; in the 2nd they were additionally fed on uninfected mice. The ticks were housed in moist boxes at 20—22 °C.

The presence of the virus in ticks was tested on day 10, 20, 30, 45 and 60 post-infection (p.i.). The sample consisted of 3 unfed or additionally fed female ticks for virus titration or indirect fluorescent antibody test (IFAT).

Mosquitoes. Laboratory colonies of *C. p. molestus*, *A. atroparvus*, *Ae. aegypti* were used. The mosquitoes were infected by feeding with virus-containing mixture through a biological membrane. The infecting mixture consisted of 20% brain suspension of albino mice and defibrinated mouse blood at 1 : 1 ratio. The titre of the virus suspension was 6.0 log LD_{50} and in the biological transmission experiment 7.0 log LD_{50}/0.02 ml. The amount of consumed virus-containing fluid was the same for all three species. The mosquitoes were kept at 27 °C and 80% relative humidity.

Virus titration in the mosquitoes and IFAT were carried out on days 7, 14, 21, and 28 post-infection. Each sample consisted of 5 female mosquitoes.

To prepare mosquito or tick suspensions, the above mentioned number of athropods were grinded in 1 ml of Hank's solution with antibiotics and centrifuged for 10 min at 2 500 rev/min under cooling. The results of the titration of mosquitoes and ticks suspensions reflect the virus content in one sample.

Karshi virus was detected in ticks and mosquitoes by standard methods: titration in newborn albino mice, IFAT and by biological transmission. Positive biological transmissions were determined by seroconversion as examined by complement fixation (CF) test.

Results

Virus reproduction in ticks

Table 1 presents the results in ticks infected with the suspension containing virus in the titre of 6.0 log LD_{50}/0.02 ml.

In the postfed *H. asiaticum* on day 20 p.i. the virus antigen was detected by IFAT in Malpighian vessels, haemolymph, and ovaries. The virus titre in the tick was then 2.0 log LD_{50}/0.02 ml. On days 30, 45, and 60 an increase in virus titre up to 4.5 log LD_{50}/0.02 ml was observed associated with the occurrence of fluorescence in the salivary glands (Figs 1 and 2).

In the postfed *D. daghestanicus* ticks the virus was detected also on day 20 p.i. The virus titre in ticks was 2.5 log LD_{50}/0.02 ml. By means of IFAT the virus was detected in the Malpighian vessels, haemolymph and ovaries. In the salivary glands the Karshi virus was found on day 45, when the virus titre in the ticks body had been 3.0 log LD_{50}/0.02 ml.

Table 1. The study of H. asiaticum and D. daghestanicus ticks experimentally infected by Karshi virus(strain Kaz-816) by titration in newborn albino mice and IFAT

Virus titre	Tick species	Feeding on albino mice	Results of titration (log LD_{50}/0.02 ml) at various times post-infection (days)					Time of appearence of the virus in salivary glands (days)
			10	20	30	45	60	
4.5	*H. asiaticum*	+	0	0	0	4.5	n	45
4.5	*H. asiaticum*	—	0	0	0	1.0	3.0	60
4.5	*D. daghestanicus*	+	0	0	0	4.5	n	45
4.5	*D. daghestanicus*	—	0	0	0	1.5	2.0	60
6.0	*H. asiaticum*	+	0	0	3.5	4.0	4.5	30
6.0	*H. asiaticum*	—	0	0	1.0	2.0	3.5	60
6.0	*D. daghestanicus*	+	0	0	2.5	3.0	n	45
6.0	*D. daghestanicus*	—	0	0‘	2.0	2.5	n	60

Note: (+) — ticks after infection fed on albino mice
(—) — ticks after infection not fed on albino mice
(n) — not titrated

In the unfed *H. asiaticum* and *D. daghestanicus* ticks, the virus was reproduced slower and reached 2.0 log LD_{50}/0.02 ml by day 45. By IFAT virus antigen was detected in the salivary glands by day 60. The titration results in ticks infected with the suspension containing virus in the titre of 4.5 log LD_{50}/0.02 ml are represented in Table 1.

In postfed *H. asiaticum* and *D. daghestanicus* ticks the virus was detected by titration in newborn albino mice and by IFAT only on day 45 p.i., i.e. at the time when the virus titre in ticks was 4.5 log LD_{50}/0.02 ml. IFAT allowed to detect the virus in the Malpighian vessels, haemolymph, ovaries and salivary glands.

Table 2. The study of C. p. molestus, An. atroparvus, and Ae. aegypti mosquitoes experimentaly infected by Karshi virus (strain Kaz-816) by titration in infant albino mice and IFAT

Mosquito species	Titration results (log LD_{50}/0.02 ml) at various intervals post-infection (days)				Appearance of specific fluorescence in salivary glands (days)
	7	14	21	28	
C. p. molestus	0	1.5	2.5	3.0	21
An. atroparvus	2.5	3.5	4.0	—	7
Ae. aegypti	1.0	3.0	3.5	—	21

Note: vitus titre in the infecting suspension during the inoculation of mosquitoes — 6.0 log LD_{50}/ /0.02 ml
(—) — not titrated

In infected unfed ticks the Karshi virus was found on day 45, but its amount was significantly smaller than in the additionally fed ticks. On day 45 p.i. the virus was detected by IFAR in the Malpighian vessels, haemolymph, ovaries and only on day 60 in the salivary glands.

Karshi virus reproduction in mosquitoes

The next experiment dealt with the reproduction of Karshi virus in mosquitoes (Table 2).

In the infected *C. p. molestus* mosquitoes the virus was found on day 14, its titre increasing from 1.5 to 2.5—3 log LD_{50}/0.02 ml by day 28 p.i. On day 14, specific fluorescence in the stomach and ovaries was observed. In the salivary glands the virus appeared on day 21 p.i.

In the *An. atroparvus* mosquitoes the virus titre was 2.5 log LD_{50}/0.02 ml already on day 7 and by day 21 p.i. it reached the value of 4.0 log LD_{50}/ /0.02 ml. Specific fluorescence was observed in the stomach, ovaries and some salivary gland cells on day 7. On day 14 and 21 intensive fluorescence appeared in the salivary gland cells indicating the active virus reproduction and accumulation.

In *Ae. aegypti* mosquitoes the virus titre was 1.0 log LD_{50}/0.02 ml on day 7 p.i. By IFAT virus antigen was detected in the stomach and ovaries. In the salivary glands specific fluorescence was observed on day 21, the virus titre in the organism of mosquitoes being 3.5 log LD_{50}/0.02 ml.

Based on the findings of IFAT and titration of tick and mosquitoe suspensions in newborn albino mice we followed the possible biological transmission of Karshi virus. Two-day-old suckling albino mice were used as susceptible animals. On day 27 p.i. the *Ae. aegypti* females were fed on infected mice (each female mosquito on one mouse). Of 9 mice on which the mosquitoes were fed, 7 became ill on day 14. Sucrose-acetone antigen was prepared from the brain of these mice for identification of the virus by CF test. The antigen reacted with immune ascitic fluid (IAF) to Karshi virus diluted 1 : 16 at a titre of 20.

Discussion

At present, plenty of evidence has been accumulated for preservation in ticks (*Ixodidae*) of mosquito-borne arboviruses, as well as for the possibility of reproduction of tick-borne viruses in mosquitoes. This can be exemplified by WN and tick-borne encephalitis (TBE) viruses which can be naturally transmitted by several mosquito and tick species. Thus, for instance, natural WN virus infection was established for 8 mosquito species and 5 tick species. Experimental WN virus infection was reported for 13 mosquito and 4 tick species. Natural TBE virus infection was established for 22 tick and 3 mosquito species, and under experimental conditions in 2 mosquito and 21 tick species (Burlakov and Pautov, 1975; Gromashevsky *et al.*, 1973, 1975; Sidorova *et al.*, 1973).

Taking into account that Karshi virus is antigenically close to WN virus, we supposed that its natural vectors can be not only numerous ticks, but also some mosquitoes.

Our data confirm the replication of Karshi virus (strain Kaz-816) in the body of *H. asiaticum* and *D. daghestanicus* ticks, the maximum virus titre being 4.5 log LD_{50}/0.02 ml. The comparison of the titration results in newborn albino mice with those of IFAT, in these 2 tick species demonstrated the similar degree of virus reproduction suggesting that *D. daghestanicus* as well as *H. asiaticum* ticks can be the vectors of Karshi virus.

Additional feeding of ticks on animals after forced dose infection (using Alekseev's equipment) significantly affects virus reproduction in the tick body. The virus is reproduced more actively, it is more rapidly accumulated in different organs and appears earlier in the salivary glands.

The experiments have shown that all 3 mosquito species *C. p. molestus*, *An. atroparvus* and *Ae. aegypti* can be infected by the Karshi virus. The virus was reproduced in different organs of mosquitoes including salivary glands. IFAT allowed to detect virus antigen in all regions of salivary glands, but their concentration was low as followed from the absence of granule formation (Janzen, Wright, 1971; Gaidamovich *et al.* 1973). At the same time the presence of virus in the proximal regions of the salivary glands adjacent to the excretory duct allows to suggest that these mosquito species may transmit the virus. The posibility was confirmed in our experiments for *Ae. aegypti* mosquitoes. The virus titre was sufficiently high in all species tested, reaching 3.0—4.0 log LD_{50}/0.02 ml on day 21 p.i. Its reproduction was especially intensive in *An. atroparvus* mosquitoes reaching the titre of 2.5 log LD_{50}/0.02 ml on day 7 p.i.

Thus, our experiments demonstrated the reproduction of Karshi virus in the body of *H. asiaticum* and *D. daghestanicus* ticks and *C. p. molestus*, *A. atroparvus* and *Ae. aegypti* mosquitoes. The presence of the virus in the organism of these arthropods was recorded over 60 days in ticks and over 21—28 days in mosquitoes (the observation period). According to IFAR findings, on day 21 Karshi virus could be detected in cells of proximal region of the lateral parts of salivary glands, which suggested the possibility of biological transmission of the virus by mosquitoes. This suggestion was supported by successful experiment of Karshi virus transmission to 2-day-old albino mice by the bite of *Ae. aegypti* mosquitoes.

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Explanation of Figures (Plate XL):

Fig. 1. Control salivary gland of *H. asiaticum* tick; magn. ×80.

Fig. 2. Salivary gland of *H. asiaticum* tick infected with Karshi virus (day 20 .pi.); magn. ×80.