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# "Karshi" Virus, a New Flavivirus (Togaviridae) Isolated from *Ornithodoros Papillipes* (Birula, 1895) Ticks in Uzbek S.S.R.

By

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With 3 Figures

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## Summary

Three identical strains of an arbovirus were isolated from 475 *Ornithodoros papillipes* ticks collected in June, 1972, in burrows of the great gerbil (Rhombomys opimus Licht., 1882) in the environs of Beshkent, Karshinsk steppe, Uzbek S.S.R. The isolate was found to range among flaviviruses. Complement-fixation, agar diffusion precipitation and neutralization tests is tissue culture and mice indicated a one-way antigenic relationship between the isolate and West Nile virus. However, the pattern of differences between them made it possible to consider the isolated agent as a new virus, "Karshi" virus. The results of electron microscopic studies of this virus are presented.

## Introduction

Development of arid areas in Uzbekistan connected with a flow of a great number of people from other regions of the U.S.S.R. caused the necessity of examining these territories for arboviruses. In June of 1972 an epizootic reconnaissance was carried out in Karshinsk steppe, Uzbek S.S.R. The present paper reports the isolation of the three strains of arboviruses from ticks collected there and their identification.

## Materials and Methods

### *Tick Collection*

Ticks were collected in Karshinsk steppe over the area of 20 km south-east of Beshkent. The climate is semi-desert, with annual precipitation of 190—300 mm pre-

dominantly falling within a cold season. An average monthly temperature (C°) are I—0°, II—5°, III—10°, IV—20°, V—30°, VI—VII—VIII—35°, IX—25°, X—15°, XI—10°, XII—5°. The sum of annual temperatures above 10° is 4860—5010° C. The soil is desert and sandy. Major vegetation is Artemisia and Salsola.

*Ornithodoros papillipes* ticks were mainly collected in burrows of a typical representative of the desert fauna — the great gerbil, Rhombomys opimus Light. 1882.

### *Isolation of Virus*

475 ticks (imago) were grouped in pools, 25 ticks in each. The ticks were transferred to dry ice for 3—5 minutes, then washed with alcohol for 2—3 minutes and 3—4 times with physiological saline with penicillin and streptomycin (500 units per 1 ml medium) for 5 minutes. After grinding, suspension was prepared by adding 10-fold volumes of Hanks' solution, pH 7.7—7.8, with 10 per cent normal rabbit serum and penicillin and streptomycin (100—200 units per 1 ml). Suspension was centrifuged at 2000 rpm for 10 minutes. The supernatant was collected and used for infection of 1—2-day-old suckling mice. Infected animals were under observation for 14 days, the brain of diseased animals was examined at autopsy. For subsequent passages, there was used 10 per cent brain suspension prepared in Hanks' solution, pH 7.6—7.8 with antibiotics. Reisolation was performed from original suspension of ticks kept at —70° C.

### *Antigens and Sera*

Antigens were prepared from brain tissue of diseased animals by sucrose-acetone extraction (1). Immune ascitic fluid (IAF) was prepared according to the technique of GAIDAMOVICH *et al.* (2).

For identification of the isolated strains, IAFs against the following arboviruses were used: Alphavirus (Sindbis), the Uukuniemi group (Uukuniemi), the Congo group (Crimean Hemorrhagic Fever), the Kemerovo group (Baku), Flavivirus (West Nile, strain IG-2266 of the Indian antigenic subgroup; West Nile, strain A-1640 of the African antigenic subgroup; Usutu, Kambodia, Koutango, Apoi, Sokuluk, Royal Farm, Japanese encephalitis, Tyuleniy, tick-borne encephalitis, Kyasanur Forest disease, Langat, looping-ill, Powassan and Negishi).

### *Serological Tests*

For serologic identification, complement-fixation (CF), neutralization (N) and agar diffusion precipitation (ADP) tests were used. The CF test was performed by a macromethod with 3 doses of complement at 4° C. The N test was carried out according to the routine technique in 2—3-week-old mice and in chick embryo cell culture. The latter reaction was evaluated by the degree of neutralization of interference with Western equine encephalomyelitis (WEE) virus. The ADP test was carried out with sucrose-acetone antigens concentrated 8—10 times with polyethylenglycol (PEG) (2). The hemagglutination (HA) test was performed by a micromethod with goose erythrocytes in the pH zones from 5.6 to 7.0 at 20° C.

### *Electron Microscopy*

For electron microscopy, brain tissue was randomly cut into blocks of 1 $mm^3$ and fixed at 4° C for 2 hours in 2.5 per cent phosphate-buffered glutaraldehyde. Tissues were then post-fixed for 1 hour in 1 per cent buffered osmium tetroxide, dehydrated in a graded ethanol series and embedded in araldite. Sections were cut with glass knives with uranyl acetate and lead citrate and examined in a JEM-100B electron microscope.

## Results

### *Isolation of Viruses*

Three strains (LEIV-2247 Uz, LEIV-2251 Uz and LEIV-2252 Uz) were isolated from pools of *O. papillipes* 24—26. X. 1972. Incubation period was 8—12 days,

when isolation attempts were made in suckling mice, in subsequent passages it decreased to 7—10 days. The titer of the virus in the brain of mice weighing 7 g (2—3-week-old) reached 9.0—9.5 log $LD_{50}$ per 0.03 ml. The illness in mice was accompanied by the development of paralyses, the animals died several hours after the appearance of the first symptoms. All strains were reisolated one month after storage at —50° C.

*Identification*

Antigen from the isolated virus regularly caused hemagglutination of goose erythrocytes in the pH zone from 6.0 to 7.0, with the optimum at pH 6.2.

According to the results of the CF test, the isolated virus strains were not antigenically related to Sindbis, Uukuniemi, CHF and Baku viruses. Polyvalent Flavivirus serum reacted with antigens under study in a titer of 1:16—1:32. The CF test with different representatives of Flaviviruses revealed an antigenic relationship between them and newly isolated viruses (Table 1). The closest relationship was observed with West Nile virus. Further studies were carried out with a prototype strain, LEIV-2247 Uz. The results of the N test in chick embryo cell culture as revealed by interference of WEE virus confirmed that the strains isolated are Flaviviruses (Table 2).

As seen from Tables 1 and 2, LEIV-2247 Uz is most closely antigenically related to West Nile virus. However, this is a one-way relation: serum against West Nile virus neutralizes LEIV-2247 Uz as homologous serum does however West Nile virus is not neutralized with serum against LEIV-2247 Uz.

Confirmation of the one-way antigenic relation between these two viruses was also obtained in cross CF and N tests in white mice (Tables 3 and 4). Since

Table 1. *Antigenic relations between Karshi virus and Flaviviruses as shown by the complement fixation test*

| Antigens | Strains of Karshi virus | | |
|---|---|---|---|
| Immune ascitic fluids | LEIV-2247 Uz | LEIV-2251 Uz | LEIV-22 Uz |
| Group B | 16 | 16 | 16 |
| LEIV-2247 Uz | 256 | 256 | 256 |
| West Nile | 64/128 | 64/128 | 32/128 |
| Usutu | 8/128 | nt | nt |
| Kambodia | 8/32 | nt | nt |
| Koutango | 8/128 | nt | nt |
| Apoi | 32/128 | nt | nt |
| Sokuluk | <8/128 | 8/128 | 8/128 |
| Royal Farm | 16/128 | nt | nt |
| Japanese encephalitis | 16/256 | 8/256 | 8/256 |
| Tyuleniy | 16/256 | 32/256 | nt |
| Tick-borne encephalitis | 32/128 | 16/128 | 16/128 |
| Kyasanur Forest disease | 32/128 | 32/128 | nt |
| Langat | 16/64 | 8/64 | nt |
| Louping ill | 8/128 | 8/128 | nt |
| Powassan | 32/128 | 64/128 | nt |
| Negishi | 32/256 | nt | nt |

Numerator: heterologous titer
Denominator: homologous titer
nt: not tested

Table 2. *Antigenic relations between Karshi virus and Flaviviruses as shown by the neutralization test in chick embryo cells culture*

| Viruses \ Sera | LEIV-2247 Uz | West Nile[a] | CHF | Langat | Looping ill | Powassan | TBE | Kadam | Virus titers per ml |
|---|---|---|---|---|---|---|---|---|---|
| LEIV-2247 Uz | 4.7[b] | 4.6 | 0.2 | 0 | 0 | 1.5 | 0 | 0 | 7.0 |
| West Nile[a] | 0.2 | 2.0 | nt | nt | nt | nt | nt | nt | 5.3 |
| Kyasanur Forest disease | 0.1 | nt | 1.4 | nt | nt | nt | nt | nt | 5.0 |
| Langat | 1.0 | nt | nt | nt | nt | nt | nt | nt | 6.5 |
| Louping ill | 0 | nt | nt | nt | 2.7 | nt | nt | nt | 6.4 |
| Powassan | 0.3 | nt | nt | nt | nt | 3.8 | nt | nt | 7.3 |
| Tick-borne encephalitis | 0.8 | nt | nt | nt | nt | nt | 1.7 | nt | 4.0 |
| Kadam | 0 | nt | nt | nt | nt | nt | nt | 1.5 | 6.3 |

[a] Strain A-1640 of African antigenic subgroup
[b] in log of neutralization index
nt: not tested

Table 3. *Antigenic relations between Karshi virus and West Nile virus as shown by checkerboard titration in the complement fixation test*

| Antigens \ Sera | LEIV-2247 Uz | West Nile[a] |
|---|---|---|
| LEIV-2247 Uz | 128/320 | 64/20 |
| LEIV-2252 Uz | 128/nt | 32/nt |
| West Nile[a] | <8/<8 | 256/320 |

Numerator: serum titer
Denominator: antigen titer
nt: not tested

[a] Strain A-1640 of African antigenic subgroup

Table 4. *Antigenic relations between Karshi virus and West Nile virus as shown by checkerboard titration in the neutralization test in mice*

| Virus \ Sera | West Nile[a] | LEIV-2247 Uz |
|---|---|---|
| LEIV-2247 Uz | 4.8[b] | 5.5 |
| LEIV-2252 Uz | 4.8 | 6.0 |
| West Nile[a] | 5.8 | 1.6 |

[a] Strain A-1640 of African antigenic subgroup
[b] in log of neutralization

Table 5. *Antigenic relations between Karshi virus and West Nile virus as shown by the agar diffusion precipitation test*

| Antigens \ Sera | LEIV-2247 Uz | LEIV-2251 Uz | LEIV-2252 Uz | West Nile (Indian) | West Nile (African) |
|---|---|---|---|---|---|
| LEIV-2247 Uz | + | + | + | − | − |
| West Nile (Indian) | − | − | − | + | + |
| West Nile (African) | − | − | − | + | + |

the ADP test is the most sensitive reaction for Flaviviruses, this method was also used for determining antigenic relationships between West Nile virus and LEIV-2247 Uz. As seen from Table 5 and Figure 1, the ADP test does not reveal antigenic relationships between these viruses.

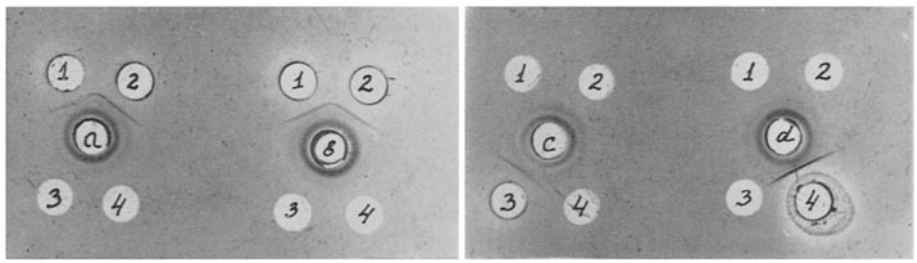


Fig. 1. Antigenic relationship between Karshi virus, West Nile virus and Powassan virus in the agar diffusion precipitation test
1. IAF to West Nile virus (A-1640), 2. IAF to West Nile virus (FG-2266), 3. IAF to LEIV-2247 Uz, 4. IAF to Powassan virus
a) antigen of West Nile virus (A-1640), b) antigen of West Nile virus (IG-2266), c) antigen of LEIV-2247 Uz, d) antigen of Powassan virus

Identity of LEIV-2247 Uz, LEIV-2251 Uz and LEIV-2252 Uz was shown in CF and ADP tests. This evidence makes it possible to consider the isolates as strains of an independent new arbovirus belonging to Flaviviruses. The virus was named "Karshi" after the place of its primary isolation.

An electron microscopic study of the brain tissue of Karshi virus-infected suckling mice has shown various Flaviviruses. Photomicrograph 2 represents a portion of a neuron containing numerous virions which are localized in vacuoles and cisterns of the endoplasmic reticulum (Fig. 2). The virions are 35—40 nm and nucleoids are about 25 nm in size. Similar data were obtained for a majority of Flaviviruses, in particular, for West Nile virus (4—5).

However, the morphogenesis of Karshi virus has some peculiarities of its own not observed in other representatives of Flaviviruses. These are formation of multilayer membrane strand (Fig. 3). It should be noted that the areas of cells, where the virions are revealed, always have such strands.

## Discussion

Karshi virus has a one-way antigenic relationship with West Nile virus. A similar one-way relationship with West Nile virus has been revealed in Usutu virus isolated from Mansonia aurites in Uganda (3). However, this is a reverse type of relationship as compared with that between Karshi and West Nile viruses. Of special interest would be studying the composition of a population of Karshi virus. It cannot be excluded that Karshi virus appeared due to antigenic drift in a genetically isolated population (dem) of West Nile virus. Adaptation of the virus to the argasid ticks *O. papillipes* led to the formation of a stable local focus of the virus which markedly changed the genetic composition of a population within a long period of time. Genetic studies may help in solving this question.

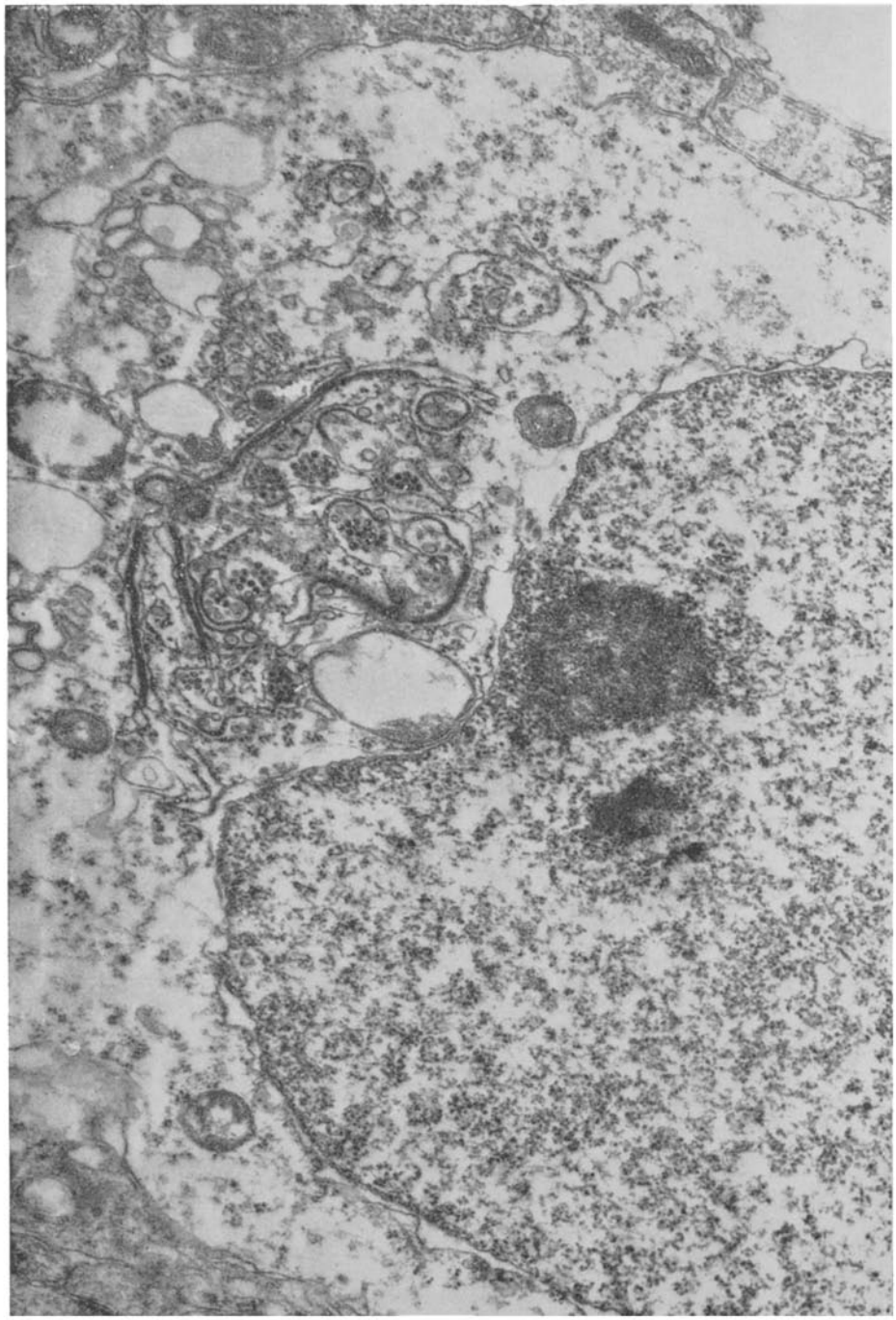

Fig. 2. A portion of the cytoplasm of a neuron in which mature virions as well as multilayed membrane strains are located in vacuoles and cisterns of the endoplasmic reticulum $\times 30{,}000$

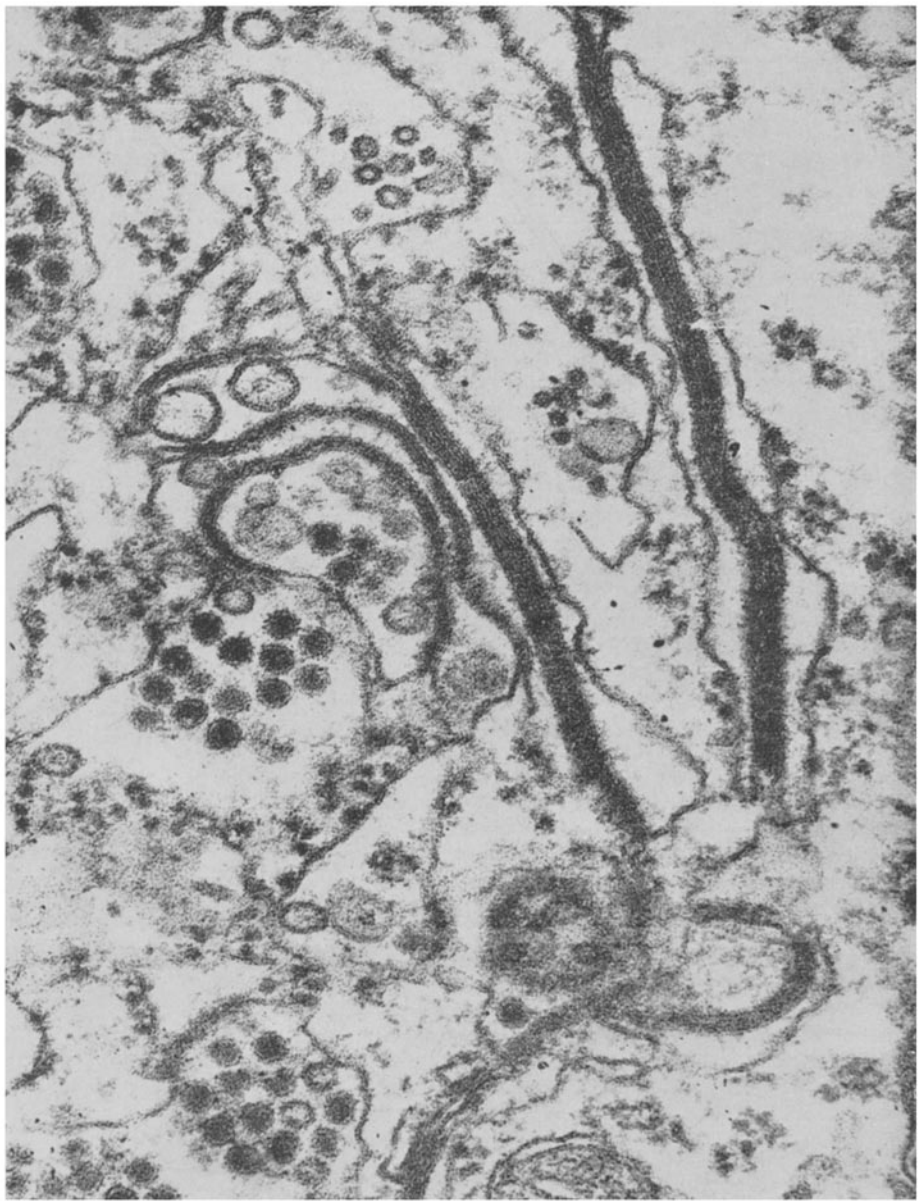

Fig. 3. The same portion at a greater magnification $\times 90{,}000$

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