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

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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 in 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 rep-
resentative 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
GAZDA~OVIC~ 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
(Crimian 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 macro-
method 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 mm³ 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-100 B 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,