SHORT REPORT: ISOLATION AND IDENTIFICATION OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS FROM A HUMAN IN PANAMA

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Abstract. Venezuelan equine encephalitis (VEE) virus was isolated from a febrile human in Panama. The patient became febrile approximately 10 days after returning from Gatun Lake in Panama. The virus was isolated from the acute phase serum and identified as VEE, subtype ID virus by monoclonal antibodies, and was confirmed by cross plaque-reduction neutralization tests.

INTRODUCTION

Venezuelan equine encephalitis (VEE) virus, family Togaviridae, genus Alphavirus, has been known to infect humans in Panama since 1961, when it was isolated from a fatal case in a 14-year-old boy residing in Canito, a village on the shore of Gatun Lake. Since this initial human case, VEE virus has been implicated as the causative agent of human illness in the geographic area of Gatun Lake. The viral subtype in each of the above cited outbreaks was found to be ID. In 1981, VEE virus was implicated in an outbreak that occurred in a group of U.S. Army soldiers undergoing training at Fort Sherman in Panama, an area also geographically located near Gatun Lake. Although the ID subtype of VEE virus was suspected as the causative agent in this outbreak, the subtype was not serologically confirmed. This report describes a more recent isolation from a febrile human and subsequent identification of VEE virus, subtype ID, which may be associated with Gatun Lake in Panama.

CASE REPORT

The patient, a 27-year-old man, was at Gatun Lake until April 10, 1993. The patient was admitted to the Gorgas Army Hospital, Republic of Panama on April 22, 1993. On the day before admission, he developed chills with a temperature of 40°C, reticular pain, headache, malaise, and backache. He denied having a sore throat, cough, nausea, vomiting, or diarrhea. He did notice the appearance of a few red spots over the upper extremities. On physical examination, the patient was alert, oriented, and cooperative. No icterus, lymphadenopathy, or conjunctival suffusion were noted. The thyroid gland was normal and the lungs were clear to auscultation and percussion. The heart had normal S1 and S2. The abdomen was soft, nontender, and without visceromegaly. There were no focal or meningeal signs. Joints were without edema or swelling. Skin examination revealed a few petechiae over the distal forearms and both hands. Laboratory tests showed a white blood cell count of 7,000/mm³, a platelet count of 243,000/mm³, and a hemoglobin level of 12.7 g/dL. On April 24, 1993, the white blood cell count had decreased to 1,300/mm³ with 18% segmented cells, 37% bands, 41% lymphocytes, and 4% monocytes. The count gradually returned to normal values, and on April 28, 1993, the total leukocyte count was 3,600/mm³. The platelet count never decreased below normal values. Urine and blood cultures were negative. Liver function test results were within normal limits and a chest radiograph was normal. The bone marrow was normocellular and all series were present. The patient was discharged on April 26, 1993.

Acute phase serum from blood drawn on April 22, 1993 was sent to the U.S. Army Medical Research Institute of Infectious Diseases for virus studies. Virus isolation was attempted by inoculating a 1:10 dilution of the acute phase serum on to a confluent sheet of VERO 76 cells grown in 25-cm² flasks. The inoculated flasks were held at 36°C and checked twice a day for cytopathic effect (CPE). Moderate CPE was seen at 72 hours post-inoculation and the supernatant was saved for further testing. Infected cells were tested against a panel of viral grouping fluids by the indirect fluorescent antibody (IFA) assay. The viral isolate (93P1513) was most reactive to the Alphavirus genus of the Togaviridae family. Subsequent IFA tests with polyclonal mouse ascitic fluids to selected alphaviruses showed that viral isolate 93P1513 was a VEE virus. Initial subtyping of viral isolate 93P1513 was by IFA tests against a series of monoclonal antibodies (provided by J. Roehrig, Centers for Disease Control and Prevention, Fort Collins, CO) as described by Roehrig and Bolin. We found viral isolate 93P1513 to be the ID subtype of VEE virus. We confirmed the subtype ID by 90% cross plaque-reduction neutralization tests (Table 1).

Before the onset of illness, the patient had been to Gatun Lake, an area of Panama associated with previous human cases of VEE caused by the ID subtype virus. The area around Gatun Lake is predominately tropical rainforest and marshy wetlands. The primary vectors for the ID subtype virus are believed to be Culex mosquitoes of the subgenus Melanoconion and small ground rodents serving as the principle reservoir hosts. This type of habitat would make control measures aimed at the vectors or reservoir hosts nearly impossible to achieve. The viral strains of the ID subtype are of particular interest since studies have indicated that

### TABLE 1

Results of cross 90% plaque-reduction-neutralization test with viral isolate 93P1513 and selected Venezuelan equine encephalitis virus strains

<table>
<thead>
<tr>
<th>VEE Strain</th>
<th>Subtype</th>
<th>Antibody</th>
<th>Trinadad</th>
<th>IC</th>
<th>E</th>
<th>68/201</th>
<th>7bV3531</th>
<th>Fe37C</th>
<th>BeAn8</th>
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</thead>
<tbody>
<tr>
<td>93P1513</td>
<td>?</td>
<td></td>
<td>640</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>40</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Trinidad</td>
<td>IAB</td>
<td></td>
<td>40</td>
<td>320</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-198</td>
<td>IC</td>
<td></td>
<td>40</td>
<td>320</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3880</td>
<td>ID</td>
<td></td>
<td>1,280</td>
<td></td>
<td>320</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>68/201</td>
<td>IE</td>
<td></td>
<td>80</td>
<td></td>
<td>320</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7bV3531</td>
<td>IF</td>
<td></td>
<td>10</td>
<td></td>
<td>160</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fe37C</td>
<td>II</td>
<td></td>
<td>10</td>
<td></td>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BeAn8</td>
<td>(Mucambo)</td>
<td>III</td>
<td>&lt;10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>320</td>
</tr>
</tbody>
</table>

<not tested>
radic outbreaks of disease caused by the epizootic strains of VEE virus may be due to mutations of the enzootic viral strains, particularly the ID subtype, which has been shown to have a close phylogenetic relationship with the epizootic strains of VEE virus.9,10

If the patient was exposed at Gatun Lake, the disease would have required an extraordinary long incubation period of approximately 10 days before onset. The usual incubation period in humans is 2–5 days. However, the length of the incubation may vary depending on the amount of virus in the inoculum.11

This report is the first isolation of VEE subtype ID virus from a naturally occurring human case in Panama since 1984.

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Disclaimer: The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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REFERENCES