

## TEN CLINICAL CASES OF HUMAN INFECTION WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS, SUBTYPE I-D\*

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**Abstract.** The clinical and laboratory findings in ten humans infected with Venezuelan equine encephalitis virus, subtype I-D, are described in this report. Clinical and laboratory data indicate that, in contrast to equine infections, human infection with these enzootic virus strains (I-D) is similar to human infection with epizootic strains (I-ABC). In most cases there was an abrupt onset of fever, muscle pain, and vomiting. Virus was recovered from sera obtained during the first 3 days of illness. Lymphopenia occurred in all patients, and neutropenia occurred in three. No sequelae of these infections were apparent.

Although human infections with epizootic strains of Venezuelan equine encephalomyelitis virus (VEE) have been reported since 1943,<sup>1</sup> and although epidemic human disease has accompanied recurrent epizootics of the disease in South America,<sup>2-4</sup> there have been few reports that adequately describe the clinical course of such disease. However, reports from Venezuela<sup>1</sup> and Texas<sup>5</sup> have emphasized the severity of such infections, particularly in children.

The major features of clinical illness caused by epizootic virus strains are sudden onset of fever or chills, myalgia and headache. Nausea and vomiting are not uncommon.<sup>5, 6</sup> White blood cell counts are frequently normal early in the infection, but the absolute lymphocyte counts are depressed.<sup>5</sup> Significant viremia is present on the 1st day of symptoms but usually disappears by the 3rd or 4th day of illness.<sup>2, 7</sup>

Some data have suggested that infection with enzootic VEE virus subtypes may be less pathogenic for man. Infection with VEE virus subtype II (Florida) may produce severe disease on

occasion,<sup>8</sup> but the majority of such infections are probably mild.<sup>9</sup> Similarly, infection with subtype III (Mucambo) caused only mild illness in 4 of 7 reported cases.<sup>10-11</sup> Experimental infection of rhesus monkeys has suggested that enzootic virus subtypes I-D and I-E are less virulent for primates.<sup>12</sup>

Although the prevalence of antibody in humans to enzootic virus strains of VEE is as high as 50% in some areas of Central America (Karl M. Johnson, unpublished data), clinical reports of infections with enzootic virus subtype I-D have been limited to one fatal case,<sup>13</sup> a short description of three laboratory infections,<sup>12</sup> and a report of a small outbreak affecting seven soldiers who camped in an enzootic focus of VEE virus.<sup>14</sup> A documented case of enzootic VEE virus infection subtype I-D in a 15-year-old boy prompted us to review our experience with this disease; ten cases of VEE virus subtype I-D infection collected over a 12-year period are included in the present report.

### MATERIALS AND METHODS

The ten patients reviewed in this study were hospitalized laboratory personnel or patients referred to us for the investigation of an acute infectious disease. Three of these patients were briefly described in a prior report,<sup>15</sup> but the other seven patients represent new cases. Two patients were females aged 32 and 34 years. The remaining eight were males ranging in age from 13 to 56 years; only two were under 20 years of age. Four of the cases represented natural infections, four cases were laboratory-acquired infections, and two

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cases could have been of either laboratory or natural origin. One man had been successfully vaccinated against VEE virus 11 months prior to his infection.

Serum, throat swab, and rectal swab specimens were obtained from most patients. Specimens of urine, saliva, vomitus, conjunctival fluid and cerebrospinal fluid were taken from smaller numbers of patients. In cases that occurred prior to 1963, swabs were expressed in 1.0 ml of Hanks' balanced salt solution containing 100 units of penicillin/ml, 100 mg streptomycin/ml, and 10% skim milk. After 1963, this medium was discarded in favor of veal infusion broth containing 0.5% bovine plasma albumin and the same concentration of antibiotics.

Each specimen was inoculated (0.02 ml/mouse) intracerebrally (ic) without dilution into one litter of suckling mice. Positive sera were titrated by ic inoculation of serial 10-fold dilutions in suckling mice; final titers were based on the number of deaths that had occurred by 72 hours. Suckling mouse intracerebral 50% lethal dose units (SMICLD<sub>50</sub>) were calculated by the method of Reed and Muench.<sup>15</sup>

All isolates were initially identified by complement fixation tests as previously described.<sup>16</sup> Nine isolates were further characterized by the short incubation hemagglutination-inhibition (SIHI) test.<sup>17</sup>

Acute and convalescent sera from each patient were tested for neutralizing (N) antibody by the plaque neutralization test in Vero cells.<sup>18</sup> Serum N antibody titers were defined as the highest dilution of serum causing 80% plaque reduction of 30-100 plaque-forming units of virus.

#### Case Report

J.J. was a 15-year-old male who experienced the sudden onset of severe diffuse headache, chills and fever 4 hours prior to admission to the hospital on 9 December 1973. It was learned that the boy had been bitten frequently by mosquitoes while spending nights on a boat moored near uprooted beds of water lettuce (*Pistia stratiotes*), a plant believed to be the breeding site of the mosquito vector of subtype I-D VEE virus.<sup>19</sup> Because of the severity of his symptoms, he was admitted to Gorgas Hospital with the differential diagnosis of leptospirosis or acute viral illness.

On admission the patient appeared acutely ill

with a temperature of 105.4°F. He was lethargic and his face and conjunctivae were suffused, but the physical examination was otherwise normal. Because he was nauseated and unable to take fluids, intravenous fluid therapy was started. Tap-water sponging and aspirin therapy were begun in an attempt to reduce his fever. During the night he vomited once. For 2 days following his admission J.J. continued to be febrile, lethargic, and nauseated. Three days after the onset of his illness he became afebrile, and his symptoms gradually disappeared although he was very weak.

Figure 1 illustrates the evolution of this patient's maximum daily temperature curve, white blood count, and serum enzyme concentrations. White blood cell differentials were not performed on the first two counts done after admission. Cerebrospinal fluid obtained on admission contained four lymphocytes and one polymorphonuclear cell per mm<sup>3</sup>; glucose and protein were normal. By the 3rd day after onset, a pancytopenia was present, and the alkaline phosphatase, lactic dehydrogenase (LDH), and serum glutamic-oxalacetic transaminase (SGOT) were all elevated. Electrophoresis demonstrated elevation of isoenzymes LDH<sub>3</sub> and LDH<sub>5</sub>. A histoplasmosis slide test and serology for leptospirosis were both negative.

On the 5th day, a repeat lumbar puncture was normal. Levels of the third component of complement (C<sub>3</sub>) done on hospital days 2, 5, 7, and 9 were 135, 125, 115 and 150 mg/100 ml, respectively. Platelets (direct method) on day 4 were 80,000/mm<sup>3</sup> and remained depressed until day 9 when the count was 300,000/mm<sup>3</sup>. Urinalysis on admission, and on days 2, 4, 6, 7, 8, and 9 were all within normal limits.

The patient continued to have a leukopenia, but by 9 days after onset, his white blood cell count had returned to 3,700/mm<sup>3</sup> with 35% neutrophils and 65% lymphocytes. The LDH and SGOT likewise were elevated until 8 days after onset when they returned to normal. At this time an epinephrine stimulation<sup>20</sup> test was done by injecting 0.5 cc of a 1:1,000 dilution of aqueous epinephrine subcutaneously. Thirty minutes after inoculation, the peripheral neutrophil count had doubled.

Nine days after admission, the patient was discharged. Two weeks later, he was completely asymptomatic and no sequelae of his illness could

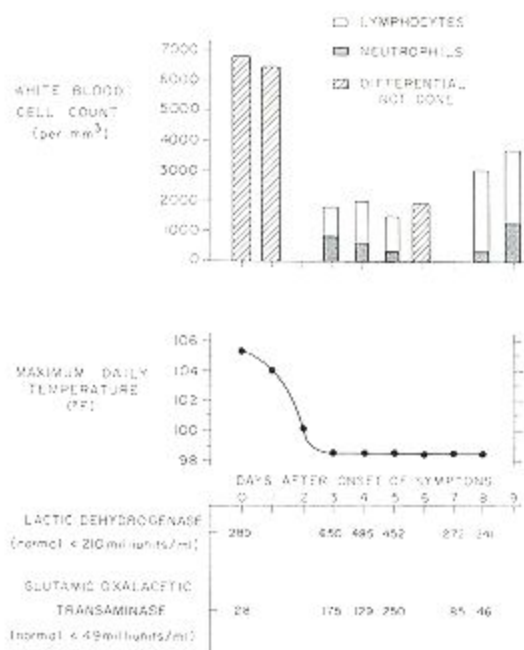


FIGURE 1. White blood cell count, lymphocyte and polymorphonuclear leukocyte counts, maximum daily temperatures, and liver enzyme values in a 15-year-old male infected with Venezuelan equine encephalitis virus, subtype 1-D.

be detected. A white blood cell count at this time was within normal limits.

An I-D strain of VEE virus was isolated from sera obtained on admission and at 1 day post-onset, and from throat swabs obtained at 2 and 4 days after the onset of illness. Virus was not recovered from serum obtained on day 2, nor from any serum thereafter.

#### Review of Other Cases

Hospital charts on seven patients and chart summaries on two patients were available for review; the prominent signs and symptoms of human 1-D VEE virus infection are summarized in Table 1. The most common presenting complaints were the triad of sudden onset of fever or chills, headache, and muscle pain. Nausea or vomiting began shortly thereafter, but other gastrointestinal symptoms such as diarrhea or abdominal cramps were infrequent. Muscle pain was frequently generalized, but was sometimes limited to back pain. When back pain was present, it was usually more severe in the lumbosacral area.

TABLE 1

Signs and symptoms of Venezuelan equine encephalitis virus subtype 1-D infection in ten human cases

Sign or symptom	Number of patients
Headache	9
Nausea or vomiting	9
Fever	9
Chills	8
Muscle or back pain	8
Lethargy	7
Sudden onset	6
Photophobia	3
Tender or palpable liver	2
Retro-orbital pain	2

Neurologic symptoms were non-specific. Seven patients were markedly lethargic for 2-4 days after onset of illness. Three patients had photophobia, and two of these also complained of retro-orbital pain. The youngest patient, a 13-year-old boy, was delirious for several hours, and a 55-year-old man had mild disequilibrium. Questionable or mild nuchal rigidity was found in two patients. One patient complained of a stinging sensation in his palms.

Erythema of the buccal cavity, flushing, or scleral suffusion was present in four patients. Three of the patients had lymphadenopathy and in two patients the lymph nodes were tender to palpation. The liver was palpable and tender in two patients, one of whom had a history of alcoholism.

In every case, the acute symptoms disappeared within 4 days of onset. Aside from asthenia and malaise that often persisted for 1-2 weeks after discharge from the hospital, there were no sequelae.

#### Laboratory studies during VEE virus infection

Figure 2 shows composite fever, white blood cell count, and white blood cell differential curves for the patients included in this study. Defervescence was rapid, and in most cases occurred within 3 days after the onset of symptoms.

In the first 24 hours after onset of symptoms of VEE, the white blood cell counts in these patients were normal or slightly elevated. White blood cell differentials of eight patients were done at the same time; 82-90% of the cells were polymorphonuclear leukocytes, and an absolute lymphopenia was present.

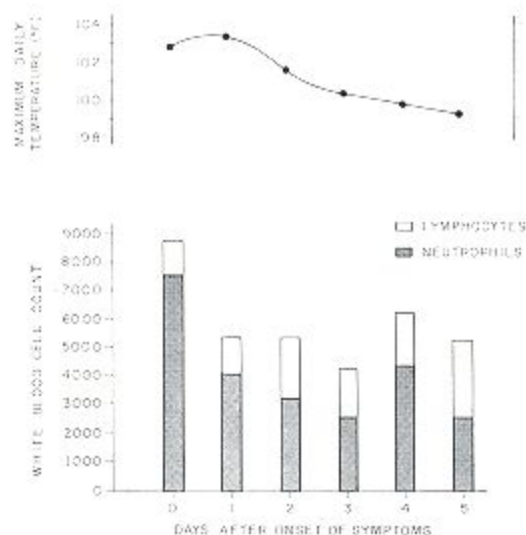


FIGURE 2. Composite curve of maximum daily temperatures, white blood cell, lymphocyte, and polymorphonuclear leukocyte counts in ten patients infected with Venezuelan equine encephalitis virus, subtype I-D.

Within 3 days of onset of symptoms, 7 of the 10 patients had developed a leukopenia; 2 patients had a combined absolute neutropenia and lymphopenia, 1 had an isolated neutropenia, and the remaining 5 patients had only lymphopenia. The magnitude of these changes was often striking; one patient had a white count of 1,400 white cells/mm<sup>3</sup> with 20 polymorphonuclear leukocytes and 23 lymphocytes counted on the smear.

In one patient, the leukopenia did not begin until 7 days after onset and, in this patient, the leukopenia was only present for 2 days. In most patients, leukopenia was of short duration, lasting only 2 or 3 days. However, in two patients it was present for longer periods of time, i.e., 4, and greater than 6 days. Lymphopenia was present in all ten patients in at least one white cell count and neutropenia was present in a total of three.

Serial platelet counts were done on two patients. One of these, a 58-year-old man, had thrombocytopenia for 4 days after onset of symptoms. Platelet counts in J.J. were also low for several days, but returned to normal after 10 days. One platelet count each was done on two other patients: one of these, done on the 6th day of illness, was low (110,000); the other, done 9 days after onset, was normal.

Only one patient had any significant changes in hemoglobin. The hemoglobin of this patient, a 28-year-old man, fell 5 g within a 1-week period. This change was not investigated.

Repeated urinalyses and determinations of blood urea nitrogen, blood glucose, electrolytes, calcium, and phosphorous were all normal. Serial measurements of SGOT were done in three patients, and determinations of the serum glutamic-pyruvic transaminase (SGPT) in two. Serial LDH determinations were done on one patient. Enzyme elevations were observed in every patient on whom tests were performed serially and lasted 4-5 days. SGPT elevations were not as marked as those seen for SGOT and LDH.

#### *Virus isolation and identification*

Virus was recovered from the acute sera of all ten patients and was identified as VEE virus by the CF test; all acute sera were drawn within 3 days of onset of symptoms. Four additional sera from three patients were obtained at times later than 3 days after onset of illness; none contained virus. Serum titers of virus ranged from 1.7 to 4.6 log<sub>10</sub> SMICLD<sub>50</sub> units/ml and, in general, sera obtained at later times after the onset of illness tended to have lower titers.

Five of seven throat swabs obtained from different patients within the first 3 days of illness contained VEE virus. In at least two patients, virus was recovered from the throat swab after the viremia had ended. At least 2.4 log<sub>10</sub> SMICLD<sub>50</sub> units/ml of VEE was present in the undiluted saliva of one patient cultured shortly after onset.

Spinal fluid was cultured from two patients; the inoculation of one obtained on the 1st day of illness caused suckling mouse deaths, but there was no record that indicated that the agent was VEE virus, and the original specimen could not be relocated. The other spinal fluid was obtained on the 5th day of illness and contained no VEE virus.

Isolates from nine patients were classified as VEE virus subtype I variant D by the SIHI test. The one case from whom no material could be located for study was from an area known to be an enzootic focus of this virus variant (Pauline Peralta, unpublished data).

Neutralizing antibody to VEE virus was present in the convalescent sera of all ten patients by 2

weeks, and in two patients bled serially, antibody appeared at 5 and 8 days after onset of symptoms.

#### DISCUSSION

The clinical course of these ten human cases of VEE virus subtype I-D infection, and the severity of the human disease caused by subtype I-ABC,<sup>4,5</sup> suggests that the virulence patterns observed in horses and rhesus monkeys do not occur in humans. Experimental infection of horses has revealed that subtype I-ABC (epizootic) strains of VEE virus cause fever and leukopenia with a high incidence of encephalitis and death, whereas virus subtypes I-D and I-E (endemic Central American strains) cause little or no equine disease.<sup>21</sup> VEE viral infection of monkeys may produce a similar pattern of virulence; subtypes I-ABC caused fever and blood enzyme elevations in rhesus monkeys whereas infection with subtypes I-D and I-E did not, even though viremias and neutropenias of similar duration and magnitude were present in all animals.<sup>12</sup>

VEE virus subtype I-D human infection closely resembled human and rhesus monkey infection with VEE virus subtypes I-ABC. Although the symptoms of virus subtype I-D infection were protean, common to virtually every case was the sudden onset of fever, muscle pain and vomiting. Complaints suggestive of neurologic involvement were not uncommon but the only neurologic finding in our cases was lethargy; this symptom often persisted for several days. Like virus subtype I-ABC infection,<sup>4,5</sup> the course of I-D infection seemed more severe in the two patients under 20 years of age. In this respect, the first isolate of VEE virus subtype I-D in Panama was made from the brain of a child who died after a short but explosive illness.<sup>13</sup> No patient in our series demonstrated a biphasic illness, although this pattern is occasionally observed in epizootic infections.<sup>22</sup> Additional data regarding the severity of pediatric infection and incidence of relapse following virus subtype I-D infection may help to differentiate these infections.

Virologic, biochemical and hematologic findings were also similar to findings in VEE virus subtype I-ABC infection of human and rhesus monkeys. Viremia could only be documented during the first 3 days of clinical illness and peak viremias occurred within the first 48 hours of illness. Virus was usually recovered from throat swabs

and, in contrast to the data from rhesus monkeys, persisted in the throat swabs of two patients for up to 48 hours after the viremia had ended.

Elevations of SGOT, SGPT and LDH occurred in those patients in whom serial determinations were performed. Lymphopenia occurred in every patient and neutropenia in three. Unfortunately, we were able to define further the nature of the neutropenia in only one patient. This patient's peripheral neutrophil count doubled following the administration of epinephrine, a response which indicates that his marginating neutrophil pool was probably intact.<sup>20</sup>

Despite the high prevalence of antibody to enzootic strains of VEE virus throughout Central America, VEE virus subtype I-D infection has been recognized only rarely. Access to medical care, or differences in the perception of illness could account for this paradox, but these hypotheses have not been tested. Although the symptoms of leptospirosis may mimic those of human VEE infection, we found that only 1 of over 100 human sera submitted for leptospirosis antibody testing contained neutralizing antibody to VEE virus. Therefore, it seems unlikely that VEE virus subtype I-D infections have been misdiagnosed as leptospirosis. A final possibility is that the sporadic occurrence of this infection, and the general absence of virologic facilities, leads to infrequent clinical recognition and laboratory confirmation of the diagnosis.

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