TONATE VIRUS INFECTION IN FRENCH GUIANA: CLINICAL ASPECTS AND SEROEPIDEMIOLOGIC STUDY

ANTOINE TALARMIN, JOCELYNE TROCCHU, JACQUES GARDON, STÉPHANE LAVENTURE, DIDIER HOMMEL, JOSIANE LELARGE, BHETTY LABEAU, JEAN PIERRE DIGOUTTE, ALAIN HULIN, AND JEAN-LOUIS SARTHOU
Centre National de Référence pour la Surveillance des Arboviroses pour la Région Antilles, Cayenne, French Guiana; Institut Pasteur de la Guyane, Cayenne, French Guiana; Intensive Care Unit, Cayenne Hospital, Cayenne, French Guiana

Abstract. Two recent cases of human infection with Tonate virus, one of which was a fatal case of encephalitis, have renewed interest in these viruses in French Guiana. The clinical aspects of confirmed and probable cases of infection with this virus indicate that it has pathogenic properties in humans similar to those of other viruses of the Venezuelan equine encephalitis complex. To determine the prevalence of antibodies to Tonate virus in the various ethnic groups and areas of French Guiana, 3,516 human sera were tested with a hemagglutination inhibition test. Of these, 11.9% were positive for the virus, but significant differences in seroprevalence were found by age, with an increase with age. After adjustment for age, significant differences were found between places of residence. The prevalence of antibody to Tonate virus was higher in savannah areas, especially in the Bas Maroni (odds ratio [OR] = 22.2, 95% confidence interval [CI] = 15.2–32.4) and Bas Oyapock areas (OR = 13.4; 95% CI = 9.8–18.4). The ethnic differences observed in this study were due mainly to differences in place of residence, except that whites were significantly less frequently infected than other ethnic groups. This study indicates that Tonate virus infection is highly prevalent in French Guiana, especially in savannah areas.

INTRODUCTION

The Venezuelan equine encephalitis complex consists of an antigenically related group of arboviruses of the family Togaviridae, genus Alphavirus. The viruses of this complex are currently classified into six antigenic subtypes by cross-neutralization and hemagglutination inhibition (HI) tests.1 Subtypes I-AB and I-C are highly virulent for horses and responsible for equine epizootics and also epidemic human disease throughout south and central America.2 The clinical symptoms in humans vary from undifferentiated fever to severe encephalitis.2 Subtype III-B (Tonate virus) was first isolated from a bird (Psarocolius decumanus) captured in 1973 in French Guiana, an overseas French possession between Brazil and Surinam.3 Thereafter, it was isolated repeatedly from various mosquitoes, especially Culex portesi, in French Guiana and Surinam.4,5 It was also recovered from pools of cliff swallow bugs (Oeciacus vicarius) and nestling birds in Colorado and Utah in 1974.6

French Guiana covers part of the Amazonian forest complex on the northeast coast of the South American continent. Ninety percent of its area of 83,500 km² is tropical rain forest; the remaining 10%, in the northern part of the country, is a coastal plain, where 90% of the 160,000 inhabitants live. The main urban centers are Cayenne, Remire-Montjoly, and Matoury, which have a total of 80,000 inhabitants. The population is made up of a large variety of ethnic groups including Creoles (50%) who are of mixed European and African descent, Amerindians (4%), Noirs Marrons (5.4%), immigrants from Haiti (20%), Brazil (4.3%), and various Asian countries (Chinese, Hmongos) (2.1%) and whites, mainly from metropolitan France (14.2%). The Noirs Marrons, an ethnic group of African origin who are descendants of slaves who escaped from Surinam during the 18th century, live mainly along the Maroni River where they have reconstructed a tribal way of life. Hmongos immigrated from Laos in 1979 and live their traditional way of life in two villages. Most of the population living on the littoral plain, and especially in Cayenne, consists of Creoles, Haitians, whites, and Brazilians. Brazilians also live along the Oyapock River with Amerindians, whereas the population along the Maroni River consists mainly of Noirs Marrons and Amerindians. Chinese are scattered across French Guiana, although most live on the littoral plain.

Although antibodies to Tonate virus are frequently found in persons living in French Guiana, little is known about the pathogenicity of this virus. It has been considered to be responsible for mild dengue-like syndromes. In 1998, two confirmed cases of infection due to Tonate virus, one of which was the first confirmed case of encephalitis, renewed interest in this pathogen.7

To describe the clinical features of Tonate virus infection and better to understand its transmission in French Guiana, we undertook a retrospective clinical study and a seroepidemiologic survey.

PATIENTS AND METHODS

Patients. All the patients included in this study had been referred to the National Reference Center for Arboviruses, Institut Pasteur de la Guyane for diagnosis of an arboviral infection. The clinical symptoms of all probable and confirmed cases of Tonate virus infection between January 1997 and June 1999 were obtained from medical files. Two confirmed cases recorded in 1975 were also included to increase the reliability of the symptomatology.

A probable case was defined as illness that was clinically compatible with arboviral infection combined with supportive serologic test results (a single convalescent-phase serum specimen containing IgM antibody for Tonate antigens and HI antibodies). To improve the specificity of this definition, we eliminated many patients who presented with IgM to Tonate virus but with clinical symptoms compatible with another viral disease (influenza-like syndrome during a flu epidemic) or biological test results compatible with bacterial disease (high neutrophil count). A confirmed case was defined as illness that fulfilled either of two diagnostic criteria: isolation of Tonate virus from serum or autopsy specimens.
or demonstration of a Tonate virus cDNA fragment by amplification from a serum sample. Cases of seroconversion from negative to positive or a four-fold or greater increase in antibody titers to viruses of the Venezuelan equine encephalitis complex in paired serum samples were considered only probable cases of infection with Tonate virus because of cross-reactions between viruses in the complex.

**Diagnosis of Tonate virus infections.** All virologic tests for arboviruses were performed at the Institut Pasteur de la Guyane, National Reference Center for Arboviruses.

**Serologic tests.** Since 1997, the serum of all patients sent for diagnosis of an arboviral infection to the National Reference Center is tested for IgM specific for three flaviviruses (yellow fever, dengue, and Saint Louis encephalitis viruses) and two alphaviruses (Tonate and Mayaro) with an IgM-capture enzyme immunoassay modified from that described previously.5 The antigens were prepared by extracting the brains of suckling mice with sucrose–acetone. HI tests for Tonate virus and tests for rheumatoid factor were performed on each positive serum to confirm the specificity of the IgM.

**Cell culture analysis and inoculation of newborn mice.** Acute-phase sera from all patients and homogenized liver and brain samples from some were diluted 10-fold in Leibovitz medium containing 3% fetal calf serum and inoculated onto subconfluent AP61 and Vero cell cultures. After seven days of culture, the cells were harvested, and the viruses were detected by monoclonal antibodies directed against yellow fever, Saint Louis encephalitis, and dengue-1, -2, -3, and -4 viruses obtained from the Centers for Disease Control (Fort Collins, CO) and mouse ascitic fluids directed against Mucambo, Pixuna, Tonate, Venezuelan equine encephalitis, Ilheus, Oriboca, Murutucu, Caraparu, Guama, Catu, Wyecomyia, Oropouche, Chagres and Punta Toro viruses obtained from the University of Texas, Medical Branch (Galveston, TX).

Clinical specimens were also inoculated intracerebrally into two-day-old suckling mice, which were observed daily for three weeks or until they died or were killed with signs of infection. If there were no signs of infection, a passage was made by intracerebral injection of a 10% centrifuged brain suspension from one suckling mouse. If signs of infection were observed, the 10% centrifuged suspension of infected brain was used to inoculate AP61 or Vero cell cultures and treated as a clinical specimen.

**Molecular detection of Tonate virus.** RNA was extracted from 130-μl aliquots of serum, brain, or liver or a supernatant of a cell culture with the QiaAmp viral RNA kit (Qiagen SA, Courtaboeuf, France) according to the recommendations of the manufacturer. Reverse transcription (RT) and the polymerase chain reaction (PCR) were performed as described previously.10 For RT, primer Venezuelan equine encephalitis 116 (5'-TACACCCAYTTRCTTTCTG-3') was used, and for the semi-nested PCR Venezuelan equine encephalitis 130 (5'-GAGAATCTGGAGCAATGTGTC-3') and 116, described by Oberste and others11 were used as outer primers, and Venezuelan equine encephalitis 130 and TonnestA (5'-GCCATGTACGGGCGTGTTA-3') were used as inner primers to amplify a portion of the PE2 glycoprotein gene.7 The 256 basepair PCR product was purified and sequenced directly with an automatic sequencing system (ACTgene; EuroSequence Gene Services, Evry, France), and the sequence was aligned with those of various previously sequenced Venezuelan equine encephalitis strains of different subtypes (obtained from Genbank).13,12 The sequences were aligned with Gene Works 2.5.1. software (IntelliGenetics, Mountain View, CA). The sequence of the new Tonate virus strain isolated in 1998 in French Guiana has been deposited in Genbank (Accession number: AF135803).

**Epidemiologic study. Sera collection.** This study was approved by the Comité Consultatif de Protection des Personnes en Recherche Biologique de Martinique. Human sera were randomly selected from several large banks of sera collected either from epidemiologic studies of human T cell lymphotrophic virus type-1, samples routinely taken from pregnant women at antenatal visits, or from the Arbovirus Laboratory collection. These sera, representative of all the ethnic groups and areas of French Guiana, were collected after informed consent was obtained between January 1992 and April 1997. All sera had been stored frozen at −80°C.

**Serologic tests.** Each serum sample was tested by HI for Tonate virus. Since sera from the arbovirus collection are sent for diagnosis of a febrile illness, HI-positive sera from this collection were also tested for IgM to Tonate virus to detect a recent infection and to avoid a bias in our results.

**Statistical analysis.** The groups were compared by the chi-square test. Odds ratios (ORs) were calculated to evaluate the association between variables and prevalent Tonate virus infection. To assess the statistical significance of associations, 95% confidence intervals (CIs) and chi-square values for trends were used.

**RESULTS**

**Clinical findings.** Between January 1997 and June 1999, six patients were considered to be probably infected with Tonate virus and two had confirmed cases. The first confirmed case (case 5) was that of a 33-year-old man who presented with moderate fever, headache, and arthralgia. A blood sample was taken on the day of onset of fever and showed a low neutrophil count (2,600/μl) and moderate thrombocytopenia (110,000/μl). The second confirmed case (case 6) was that of a two-month-old boy from Saint-Georges (Figure 1), who was hospitalized four days after the onset of fever in the intensive care unit of the Cayenne General Hospital for fever and generalized status myoclonicus.7 Despite specific treatment (diazepam, Phenobarbital, and ventilation), the patient’s neurologic condition continued to deteriorate, and he died 72 hr after admission. At the time of admission, the laboratory findings showed anemia and a high leukocyte count. The cerebrospinal fluid contained 100 leukocytes/mm3 (neutrophils/lymphocytes = 40/60 per mm3); the glucose and protein concentrations were normal (Table 1). The results of all direct and serologic tests for parasitic, bacterial, and viral infections were negative except for the presence of IgM to Tonate virus.3

In the two confirmed cases, we were unable to isolate the virus by inoculating cell cultures or newborn mice intracerebrally with clinical specimens. However, the virus was detected by molecular techniques in a blood sample from the patient who showed seroconversion to Tonate virus and in brain biopsies from the patient with encephalitis. The viruses were identified by direct sequencing of the PCR products...
and found to be more closely related to the reference strain CanAn410d (1.5% nucleotide divergence for the strain responsible for encephalitis and 3.4% nucleotide divergence for the strain responsible for a dengue-like syndrome) than to any other virus in the Venezuelan equine encephalitis complex.

Two other patients seroconverted to viruses of the Venezuelan equine encephalitis complex, but the virus could not be isolated or detected by RT-PCR. The clinical symptoms and the results of unspecific biological and virologic tests for all probable and confirmed cases are shown in Table 1. Apart from case 6, no neurologic symptoms were recorded. Most patients presented with fever, headache, myalgia, and arthralgia; mild digestive symptoms were also reported. Low neutrophil counts were recorded in four patients, and moderate thrombocytopenia (< 150,000 platelets/l) in five of the seven patients for whom the relevant data were available (Table 1). The areas of the country where the patients were presumed to have been infected with Tonate virus are presented in Figure 1.

**Epidemiologic study.** A total of 3,516 sera (from 1,822 women and 1,694 men, mean ± SD age = 28.2 ± 15.5 years, range = 1–95 years) were tested for antibodies to Tonate virus. HI antibodies without IgM were found in 418 (11.9%) sera (Table 2). IgM to Tonate virus was detected in 39 sera from patients sent for diagnosis of an arboviral dis-

---

**TABLE 1**

Clinical symptoms and biologic results in patients (all male) infected with Tonate virus*

<table>
<thead>
<tr>
<th>Case</th>
<th>Onset (mo/yr)</th>
<th>Age (yr)</th>
<th>Fever</th>
<th>Headache</th>
<th>Myalgia</th>
<th>Arthralgia</th>
<th>Digestive symptoms</th>
<th>WBC 10⁹/l</th>
<th>Plt 10⁹/l</th>
<th>Ne 10⁹/l</th>
<th>Ly 10⁹/l</th>
<th>Diagnosis of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7/97</td>
<td>25</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>2.1</td>
<td>86</td>
<td>1.4</td>
<td>0.3</td>
<td>Probable case (HI + IgM)</td>
</tr>
<tr>
<td>4</td>
<td>9/97</td>
<td>34</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>4.1</td>
<td>149</td>
<td>2.4</td>
<td>1.1</td>
<td>Probable case (HI + IgM)</td>
</tr>
<tr>
<td>5</td>
<td>2/98</td>
<td>33</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>2.6</td>
<td>110</td>
<td>1.4</td>
<td>1.1</td>
<td>Confirmed case (PCR + SC)</td>
</tr>
<tr>
<td>7</td>
<td>8/98</td>
<td>57</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>4.5</td>
<td>265</td>
<td>1.5</td>
<td>2.7</td>
<td>Probable case (SC)</td>
</tr>
<tr>
<td>8</td>
<td>3/99</td>
<td>32</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>3.5</td>
<td>134</td>
<td>1.6</td>
<td>1.3</td>
<td>Probable case (HI + IgM)</td>
</tr>
<tr>
<td>9</td>
<td>4/99</td>
<td>48</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td>4.3</td>
<td>123</td>
<td>3.6</td>
<td>0.4</td>
<td>Probable case (HI + IgM)</td>
</tr>
<tr>
<td>10</td>
<td>9/99</td>
<td>24</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td>5.5</td>
<td>121</td>
<td>4.9</td>
<td>0.2</td>
<td>Probable case (SC)</td>
</tr>
</tbody>
</table>

* WBC = white blood cells; Plt = platelet count; Ne = neutrophil count; Ly = lymphocyte count; SC = seroconversion; HI = hemagglutination inhibition; PCR = polymerase chain reaction. ? = not known.
† No neurologic symptoms were observed, except for Case 6 (encephalitis).
‡ Hemoglobin level was normal, except for Case 6 (7.3 g/L).
§ Age = 2 mo.
ease, and these patients were therefore eliminated from the study. The infection rates of women (5.8%) and men (6.8%) were similar. The seroprevalence increased significantly with age ($P < 0.001$, by trend test), with a substantial increase between the age groups < 10 and 10–19 years old. An age > 10 years was associated with a significant risk for Tonate virus seropositivity ($OR = 9$, 95% CI = 1.6–50). The seroprevalence rate differed significantly according to the place of residence ($P < 0.001$). The lowest rate was found in Cayenne and Kourou, as expected, and in the Haut-Maroni and Haut-Oyapock areas. The highest rates were found in savannah areas, especially in the Bas-Maroni and Bas-Oyapock regions. Differences in seroprevalence among ethnic groups were also significant ($P < 0.001$) (Table 2 and Figure 1). Because the association between place of residence and ethnic group is very strong in French Guiana, the differences between ethnic groups could be ascertained only in Cayenne. Whites had significantly lower rates of Tonate virus infection than other ethnic groups, but no difference was found between Creoles and Brazilians living in Cayenne (Table 3).

**DISCUSSION**

Although dengue viruses are the predominant arboviruses in French Guiana, other arboviral diseases may occur in this Amazonian country. Many of the clinical symptoms are not characteristic of a particular disease, and Tonate virus infection in particular may well be mistaken for dengue fever. The clinical findings indicate that Tonate virus is, like other viruses of the Venezuelan equine encephalitis complex, responsible for dengue-like syndromes. However, one case of encephalitis due to this virus was described recently (case 6), and another case of encephalitis due to an unidentified virus of the Venezuelan equine encephalitis complex was recorded in a young girl in 1993, although infection with Tonate virus could not be confirmed. The clinical features of infection with Tonate virus are similar to those described for other viruses of the Venezuelan equine encephalitis complex. During human epidemics with subtypes IAB and IC, encephalitis is rare. Infections with other subtypes are less well documented, but the symptoms seen with Mucambo viruses and subtype ID seem to be similar. Therefore, Tonate virus appears to be as pathogenic as other viruses of the complex and should not be considered, as it used to be, responsible for only mild dengue-like syndromes.

Our seroepidemiologic study indicates that human infections with viruses of this complex, and especially Tonate virus, are frequent in French Guiana. Tonate virus has been isolated from mosquitoes and animals more often than Mucambo and Cabassou viruses. Between 1973 and 1977, Tonate was isolated from 69 mosquito pools and 15 birds, whereas Cabassou was isolated from 13 mosquito pools and five vertebrates and Mucambo from only one mosquito.
Moreover, Tonate virus is the only one of the complex that has been isolated from humans in French Guiana.

Despite the high seroprevalence, this virus has been isolated from humans only twice since 1997. The reasons for this discrepancy could be a short duration of the viremia and/or a low viral load. Sensitive techniques such as RT-semi-nested PCR may allow a higher rate of recovery of the virus. The areas of transmission of Tonate virus could also explain the low rate of isolation of this virus in humans. The highest rates of antibodies were found in remote savannah areas, especially in isolated regions such as Bas-Maroni and Bas-Oyapock. The transport of biological samples to diagnostic laboratories is thus difficult, and physicians rarely ask for arbovirus serology, with only severe cases, such as that of encephalitis, being sent for diagnosis.

Differences in place of residence explain the differences observed between ethnic groups, since in French Guiana many ethnic groups live in single-ethnic villages. The seroprevalence was high in Amerindians and Brazilians, most of whom live in Bas-Oyapock and Bas-Maroni. The rates of Brazilians and Creoles living in Cayenne were similar. Only whites, many of whom have lived in French Guiana for only a few years and have therefore had shorter exposure to the vector, had significantly lower seroprevalence rates (Table 3).

Differences in seroprevalence with place of residence may also be due to differences in the distribution of the reservoir or of the vector, which is not known for Tonate virus in French Guiana. Rodents appear to serve as the reservoir for most viruses of the Venezuelan equine encephalitis complex. Although Tonate virus has not been recovered from rodents, possibly because of the small number tested, antibodies to this virus were found in 23% of Proechimys spp. and more than 1,100 marsupials tested, giving a prevalence of 2.5–6.2% according to species.

In the 1970s, Tonate virus was isolated mainly from Culex spp. and especially from C. portesi, which appeared to be the main vector. This strain is frequent in savannah and coastal areas of French Guiana, even near towns; it has also, although rarely, been found along the Maroni River. It has never been found in the Haut Oyapock region. Hudson reported that in Surinam, which is similar to French Guiana, C. portesi is found only in savannah regions and never in the rain forest. The distribution of this vector could therefore explain the lower rates observed in the inner parts of the country where the rain forest is very dense. Further study of the distribution of this vector in French Guiana could confirm this hypothesis.

**REFERENCES**

6. Monath TP, Lazzuick JS, Cropp CB, Rush WA, Calisher CH, Kinney RM, Trent DW, Kemp GE, Bowen GS, Francy DB, 1980. Recovery of Tonate virus ("Bijou-Bridge" strain), a member of the Venezuelan equine encephalomyelitis virus complex, from cliff swallow nest bugs (Oecocerus vicarius) and...