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Short Communication

Genotypes of clinical varicella-zoster virus isolates from Manaus, Brazil

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Abstract

Introduction: Vaccination against varicella-zoster virus (VZV) has been effective and safe in countries that routinely administer the vaccine. Brazil began universal VZV vaccination in 2013. This study aimed to identify VZV genotypes present in Manaus, Brazil prior to widespread immunization. **Methods:** Vesicular lesions or cerebral-spinal-fluid samples were collected from patients diagnosed with VZV, herpes zoster, or meningitis/encephalitis. DNA was extracted, amplified, and sequenced. **Results:** Half the isolates were clade-5 viruses and the remaining were divided between the European clades 1 and 3. **Conclusions:** This study provides insights into the circulating VZV genotypes in Manaus prior to widespread vaccination.

Keywords: Genotypes. Varicella-zoster virus. Manaus.

Varicella-zoster virus (VZV) is a human herpesvirus of the subfamily, Alphaherpesvirinae. Primary infection with VZV causes chickenpox (varicella), a typically mild, febrile, rash illness; it may, however, be complicated by pneumonia, meningitis, or bacterial superinfections[1,2]. Latent infection is established in dorsal-root ganglia during VZV infection, which can reactivate to cause zoster, a dermatomally-distributed, often severe, rash illness[1].

In Brazil, 2,334 deaths were attributed to VZV during the pre-vaccination period from 1996 to 2011. Approximately 67% occurred among children <9 years old. There were 62,052 hospitalizations due to VZV, primarily in children <9 years old, with >27% (~17,000) occurring in children aged 1-4 (DataSUS: http://www2.datasus.gov.br/DATASUS). Little data about circulating VZV genotypes in Brazil exists. Two published reports included data on Brazilian isolates[3,4], but used less robust methods than are currently available. Loparev and coworkers provided early evidence of recombination in VZV strains collected in Mexico and Chile, then defined as genotype M (mosaic)[4].

Varicella vaccination has been effective and safe in countries that routinely administer the vaccine; a 2-dose US schedule of VZV vaccine prevented 87.5% of clinically-diagnosed VZV infections, and 97.3% of laboratory-confirmed VZV[5]. Brazil began universal VZV vaccination in 2013 through the National Immunization Program. The VZV genotypes identified in this sample provide a baseline of circulating strains in Manaus, Brazil prior to routine immunization against VZV. As vaccination rates increase, we anticipate that reduced rates of transmission will drive a shift in the circulating VZV genotypes, as has occurred during other vaccination programs.

During February–September 2013, vesicular-lesion or cerebrospinal-fluid (CSF) samples for VZV genotyping were collected from a total of 14 hospitalized patients with clinically diagnosed varicella, herpes zoster, or meningitis/encephalitis. All samples were from patients in the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, a tertiary and public healthcare center located in the city of Manaus. The meningitis and encephalitis patients included in the study were admitted between February and September 2013.

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Case data were collected from the hospital's electronic database and from inpatient medical records. The criteria for inclusion in this series were patients with a diagnosis of VZV confirmed by PCR using cerebrospinal fluid (CSF) or skin lesions. This study was approved by the Ethical Review Board of the FMT-HVD # 43123315.2.0000.0005 and is in keeping with the Helsinki Declaration of 1964, as revised in 1975, 1983, 1989, 1996, and 2000. Written informed consent was obtained from all the patients included in the study or from their legal guardians.

DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR amplification and sequencing of three VZV amplicons for ORF21 (positions 33725 and 33728), ORF22 (positions 37902, 38055, 38081 and 38177), and ORF50 (position 87841) (**Figure 1**) were used to distinguish wildtype VZV clades designated in 2010[6] as previously described[7]. DNA amplification was performed in a GeneAmp PCR 9700 thermal cycler (Applied Biosystems, Grand Island, NY), using the AmpliTaq Gold 360 MasterMix (Life Technologies, Grand Island, NY) in 50-µl reaction volumes. PCR conditions were 1 cycle at 95°C for 10 min, 40 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and 1 cycle at 72°C for 10 min. PCR products were cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA) and added to the cycle-sequencing reaction (20 µl). Cycle-sequencing conditions were 25 cycles at 96°C for 10 s, 50°C for 5 s, 60°C for 4 min, followed by 72°C for 7 min. These products were treated with CleanSeq beads (Beckman Coulter, Indianapolis, IN) and denatured. Denatured samples were sequenced on the ABI 3500 genetic analyzer (Applied Biosystems), and sequences analyzed using Sequencher 5.4.6 software (Gene Codes Corporation, Ann Arbor, MI). All of the samples contained wildtype VZV DNA (using vaccine: wildtype discrimination)[7]. Seven isolates were determined to be clade 5 viruses (50%), 5 were clade 1 (36%), and 2 were clade 3 (14%) (**Table 1).** Depletion of the samples precluded the use of the recently-published revisions to our SNP-based genotyping method[8]. The method used fails to distinguish clade 6 from clade VIII, but since no clade 6/VIII viruses were identified, it was not relevant to this study. These data, together with patient data, are shown in **Figure 2**.

The average age of the 14 patients with test samples was 20 years (range: 1–79 years). There were 7 males, and the average duration of illness was 6.7 days (range: 4–10 days). All of the patients were citizens of Manaus with the exception of one from Manacapuru, also located in the state of Amazonas. Eight of the 14 patients were infected with VZV and 2 with herpes zoster. The most common symptoms were fever and headache. Of those patients with varicella, five children under the age of 10 presented lesions complicated with bacterial superinfection. They were not treated with acyclovir but were treated with antibiotics to control the strep infection. A generalized varicella rash was present in 6 out of the 14 patients. Herpes-zoster rash was present in two patients with vesicular rashes involving the trigeminal nerve. The remaining patients had VZV DNA detected in samples of CSF.

Among the six patients with CNS viral infection, only two developed rash, and all presented with headache, fever, vomiting and photophobia. The CSF was inflammatory with pleocytosis (>5 cells/mm3) in all patients (four cases of meningitis and two cases of encephalitis). The mean CSF white-blood-cell (WBC) count was 152.5 cells/mm3 (range: 16–320 cells). The CNS patients had lymphocyte-predominant pleocytosis with a mean of 98% lymphocytes. All patients had hyperproteinorrachia (>45 mg/dL). The mean CSF protein level was 105 mg/dL (range: 48-161 mg/dL). The mean CSF glucose was 51 mg/dL (range: 33-60 mg/dL). All of the patients with CNS infections were treated with intravenous acyclovir. All patients recovered with no sequelae on follow up.

Two patients were HIV positive. The first, a 13-year-old boy, presented disseminated lesions throughout the body and his CD4-T-cell count was 83 cells/µL. The second HIV patient was a 31-year-old male with clinical signs of meningitis, a CD4-T-cell count of 140 cells/µL, and an undetectable viral load. They both responded well to acyclovir and had an uneventful recovery. They had no other opportunistic infections and there was no VZV vaccine history information.

Sequencing data from the VZV isolates reported here showed that all the identified genotypes can be involved in

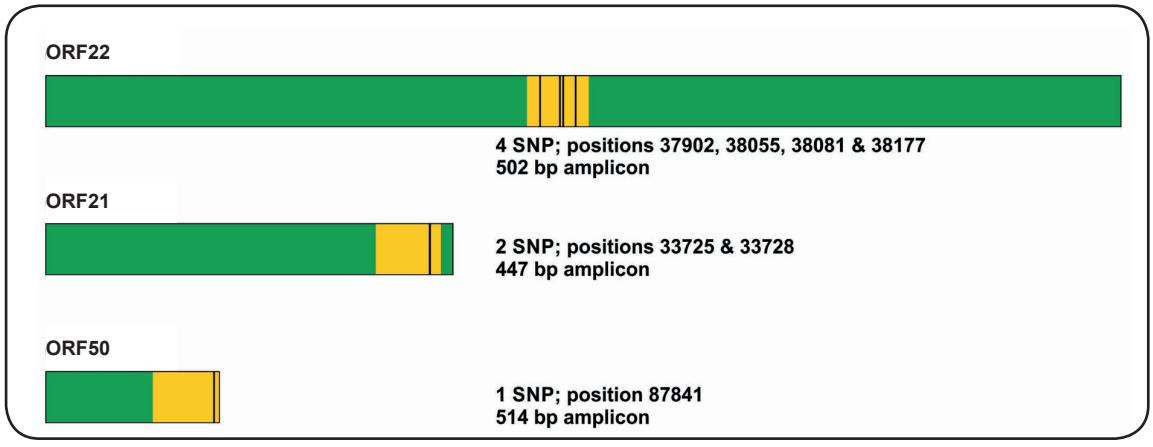

FIGURE 1: Target SNPs for the 3-amplicon method. Schematics of the ORF21-, ORF22-, and ORF50-coding regions. The regions amplified are shown in yellow and the relative positions of the targeted SNPs are indicated by black lines.

TABLE 1: Individual clinical and laboratory features of the 14 patients with varicella-zoster-virus infection.

N.	Diagnosis	Age year	Gender	Days of hospitalization	Clinical Feature	CSF WBC Count (cell/mm^3)	Specimen	Genotype
1	Varicella	13	M	10	Fever	N/A	Scab	Clade 3
2	Herpes Zoster	14	M	9	Severe Zoster Neuralgia	N/A	Scab	Clade 1
3	Herpes Zoster	36	F	8	Severe Zoster Neuralgia	N/A	Scab	Clade 1
4	Varicella	4	F	4	Fever, headache	N/A	Scab	Clade 5
5	Varicella	8	F	Not Known	Headache	N/A	Scab	Clade 5
6	Varicella	2	F	5	Fever	N/A	Scab	Clade 5
7	Varicella	3	M	4	Fever	N/A	Scab	Clade 5
8	Varicella	1	M	5	Fever	N/A	Scab	Clade 5
9	Meningitis	31	M	6	Headache, fever, vomiting, photophobia	320	CSF	Clade 5
10	Encephalitis	79	F	7	Headache, focal neurological signs	16	CSF	Clade 1
11	Encephalitis	57	M	7	Headache, focal neurological signs	120	CSF	Clade 3
12	Meningitis	7	F	5	Headache, fever, vomiting	123	CSF	Clade 1
13	Meningitis	18	M	8	Fever, headache, photophobia	16	CSF	Clade 1
14	Meningitis	18	F	9	Headache, photophobia, vomiting.	320	CSF	Clade 5

CSF: Cerebrospinal Fluid; **WBC:** white blood cell; **N/A:** Not Applicable.

ORF	21		22				50
Position*	**33725**	**33728**	**37902**	**38055**	**38081**	**38177**	**87841**
Clade 1	T	T	A	T	A	G	C
Clade 2	**C**	**C**	**G**	**C**	**C**	**A**	**T**
Clade 3	**C**	**C**	A	T	A	G	**T**
Clade 4	**C**	**C**	A	**C**	**C**	**A**	**T**
Clade 5	**C**	**C**	A	T	**C**	G	**T**
Clade 6	**C**	**C**	A	T	**C**	**A**	**T**
Clade VIII	**C**	**C**	A	T	**C**	**A**	**T**
Clade 9	**C**	**C**	A	**C**	A	G	**T**

*Genome sequence positions are based on the clade-1 reference strain, Dumas (Accession #: NC_001348.1). Differences from the clade-1 reference strain are in bold print and highlighted in yellow.

FIGURE 2: Genotyping profile using the 3-amplicon method.

CNS disease and that in the pediatric patients presented, clade-5 viruses predominated. The classifications of these samples are similar to those found in the US except that European clades (clades 1 and 3) predominate, followed by clade 5 (CDC, unpublished observation). VZV molecular epidemiology varies by geographical region, likely reflecting climatic conditions and the geographic isolation of populations, although this trend does not hold in countries with widespread immigration[6-10]. Clade 5 is the dominant genotype in Africa, and its dominance in Brazil may reflect large influxes of people from sub-Saharan Africa. The only other substantial study of VZV isolates in South America was conducted in temperate Argentina, in which exclusively European-type viruses were identified[9].

Monitoring of VZV genotypes in a susceptible population is important as a means of determining the transmission dynamics and global dissemination of the virus. As VZV vaccination is adopted and more broadly implemented worldwide, the distribution of globally-circulating varicella clades is expected to shift. Studies of various genotypes in the context of diseases may also help to identify risk factors for complications in varicella and zoster, such as neurological disease, pneumonia, bacterial superinfection, and post-herpetic neuralgia. The distribution of VZV clades in South America has been the least investigated of all the populated continents. Broader studies in South American countries should add valuable information about transmission patterns and global trends for this common virus infection.

Conflict of Interest: The authors declare that there is no conflict of interest.

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