Laboratory surveillance of dengue virus in Central Brazil, 1994–2003

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Abstract

Background: In Brazil, dengue endemic and epidemic patterns indicate an upward trend in incidence and hospitalization in the past decade. Objective: To report dengue circulating serotypes from 1994 to 2003 and the role of distinct serotypes on dengue clinical outcomes in Central Brazil. Methods: Virological surveillance for dengue cases was conducted in the city of Goiania (∼1,200,000 population) from 1994 to 2003. Samples were tested using dengue IgM antibody (MAC–ELISA) and/or virus isolation. Circulating subtypes and genotypes were identified by reverse transcriptase PCR (RT-PCR) and by restricted site-specific PCR (RSS-PCR) patterns in selected samples. Results: Adults (87.4%) were the most affected group and dengue fever accounted for the majority of the cases. Laboratory surveillance identified mainly DEN 1 serotype from 1994 to 2002 shifting to a high circulation of DEN 3 in 2003. The ratio of dengue fever to dengue with complications/DHF remained constant following the introduction of DEN 3. Diagnosis of dengue was confirmed in ∼50% of the suspected cases enhanced by RT-PCR. RSS-PCR patterns for DEN 1 and DEN 3 corresponded to the circulating subtypes in the country. Conclusions: The result of virological surveillance did not suggest a major role of infecting DEN 3 serotype in increasing disease severity during its first-year spread in Central Brazil. © 2006 Elsevier B.V. All rights reserved.

Keywords: Dengue; Surveillance; Clinical; Virus isolation; Reverse transcriptase-polymerase chain reaction; Diagnosis

1. Introduction

Dengue fever and dengue hemorrhagic fever (DHF) are considered among the most important vector-borne diseases in tropical regions, with an estimated annual incidence of 50–100 million cases of dengue fever and more than 500,000 reported cases of DHF and dengue shock syndrome (DSS) worldwide (Gubler, 1997; Guzman and Kouri, 2003). In Brazil, the introduction of DEN 1 (1986), DEN 2 (1990) and DEN 3 (2000) occurred in the southeast region, considered the main route for the spread of dengue virus countrywide (Nogueira et al., 2001; Miagostovich et al., 2002; Siqueira et al., 2005). During the last decade, there was an upward trend in incidence, peaking with more than 300 cases per 100,000 inhabitants in the year 2002, with explosive epidemics in many large cities (Siqueira et al., 2005).

Dengue fever (DF) is mainly a mild acute febrile disease and DHF/DSS is characterized by fever, thrombocytopenia, hemorrhagic manifestations, and excessive capillary permeability that may progress to shock and death (WHO, 1997). Increased risk for severity of dengue outcomes are likely to be explained by a secondary infection due to a different serotype, or by correlation with virus virulence yielded by genetic variation (Halstead, 1997; Holmes and Twiddy, 2003). The role of distinct serotypes and/or previous infection on the severity of clinical outcomes has not been fully established (Endy et al., 2004). In Brazil, increasing rates of hospitalization due
to DHF or severe DF cases, and increases in the number of deaths caused by the infection have been reported (Siqueira et al., 2005).

In the current study, we present the results of a laboratory surveillance conducted in the city of Goiania in Central Brazil since dengue virus introduction in 1994–2003. We focused on the circulating serotypes, its temporal association with the outbreaks and the severity of the clinical cases. We also present the results of a pro-active surveillance to identify serotypes by reverse transcriptase-polymerase chain reaction (RT-PCR), and the genetic characterization by RSS-PCR in selected samples. These findings are important to characterize the epidemiologic pattern of the first decade of dengue/DHF in one of the most populated cities in Central Brazil.

2. Methods

2.1. Surveillance and study site

Dengue/DHF is a reportable disease and the official Brazilian surveillance system was described in detail previously (Siqueira et al., 2005). Dengue is reported according to severity: (1) dengue fever, (2) dengue with complications, (3) dengue hemorrhagic fever (DHF) and (4) dengue shock syndrome (DSS). Dengue with complications includes the cases that do not fulfill the DHF criteria and the classical dengue case definition is inadequate due to the severity of the clinical manifestations, as follows: hemorrhagic or neurological manifestations, cardio-respiratory dysfunction, hepatic failure, thrombocytopenia (platelet counts < 50,000 per μL), leucopenia (<1000 cells) or death (Ministério da Saúde, 2005). DHF and DSS case definition follows standard criteria (WHO, 1997).

Laboratory surveillance was initiated since the first epidemic in 1994 in the city of Goiania (~1,200,000 population), in Central Brazil. Suspected cases were from the main public infectious disease reference hospital and outpatient clinics covering the metropolitan area. Dengue incidence is estimated by case report from the surveillance system. Cases were routinely confirmed by serological test or virus isolation at the regional reference laboratory. Data on age, sex, disease classification, and disease outcomes for the reported cases were extracted from digital files from the official information system.

Serological tests were performed for serum collected after 5 days of the onset of symptoms using in-house dengue IgM antibody capture MAC–ELISA (Kuno et al., 1987). The Dengue Control Program recommends serological test to all suspected dengue case in non-epidemic periods. During epidemics, around 10% of the suspected cases should be tested by IgM serology (Ministério da Saúde, 2005). From 1994 to 2003, blood samples (<7 days after the onset of disease) were obtained from suspected dengue cases from hospitals and health centers throughout the year. Virus isolation was performed at the reference laboratory (LACEN-Go, Brazil) using a monolayer of C6/36 Aedes albopictus cells (Igarashi, 1978). Dengue virus isolates were identified by an indirect fluorescent antibody test using serotype-specific monoclonal antibodies (Gubler et al., 1984).

2.2. Pro-active dengue surveillance

From May to December 2003, disease surveillance was enhanced to monitor circulating subtypes by adding molecular techniques. Eighty-seven suspected dengue cases had paired samples from acute and convalescent-phase (~day 14) collected. Aliquots of blood were immediately stored in liquid nitrogen and transported weekly to the reference laboratory for virus isolation and molecular testing. Besides routine dengue IgM antibodies, IgM/IgG antibodies were assessed by EIA (PanBio Indx, Inc., Baltimore, MD). Serology and virus isolation and reverse transcriptase PCR (RT-PCR) were performed for detecting the infecting serotype, and 12 viral isolates were analyzed by restriction site-specific-polymerase chain reaction (RSS-PCR) for genetic subtype.

The infecting DEN serotype was identified from acute-phase serum samples (n = 87) by RT-PCR (Lanciotti et al., 1992). RNA was extracted according to standard protocol (Boom et al., 1990). Molecular subtyping of DEN 1, DEN 2 and DEN 3 were performed according to previously described techniques by using RSS-PCR (Harris et al., 1999; Miagostovich et al., 2000).

2.3. Statistical analysis

Descriptive statistics for the study population characteristics and laboratory findings were performed using SPSS Inc., Chicago, IL, Version 10. χ² test was applied for comparisons between proportions and p < 0.05 considered to be significant. The study received the approval of the local Ethical Committee, and all patients or guardians gave informed consent for molecular techniques, which are not part of the routine surveillance.

3. Results

A total of 5553 samples from suspected dengue cases were tested for virus isolation from 1994 to 2003. DEN 1 was the only detected serotype until 1999 followed by virus isolation of DEN 2 and DEN 3 in 2002. The first confirmed DHF occurred in 1998.

During 2002, dengue virus was isolated in 22.8% out of 3052 samples tested. DEN 1 was isolated in 20.7% (632/3052) of the specimens being the predominant serotype among the positive isolates (90.7%), followed by DEN 2 (6.6%) and DEN 3 (2.7%). In 2003, dengue virus was isolated in 16.0% out of 431 samples tested. The predominant serotype was DEN 3 representing 76.8% of positives samples, followed by DEN 1 (17.4%) and DEN 2 (5.8%). There was
Table 1
Serotypes of dengue virus isolated, Goiania-Central Brazil, 1994–2003

<table>
<thead>
<tr>
<th>Years Reported</th>
<th>Positive/totala (%)</th>
<th>Serotypesb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DEN-1</td>
<td>DEN-2</td>
</tr>
<tr>
<td>1994–1998</td>
<td>3 (100.0)</td>
<td>–</td>
</tr>
<tr>
<td>1999</td>
<td>14 (82.4)</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>2000</td>
<td>18 (78.3)</td>
<td>5 (21.7)</td>
</tr>
<tr>
<td>2001</td>
<td>160 (78.8)</td>
<td>43 (21.2)</td>
</tr>
<tr>
<td>2002</td>
<td>632 (90.7%)</td>
<td>46 (6.6)</td>
</tr>
<tr>
<td>2003</td>
<td>12 (17.4%)</td>
<td>4 (5.8)</td>
</tr>
</tbody>
</table>

Table 2
Characteristics of dengue cases according to serotypes, 2002–2003

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Year 2002</th>
<th>Year 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 632 (%)</td>
<td>n = 46 (%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–10</td>
<td>35 (5.5)</td>
<td>2 (4.3)</td>
</tr>
<tr>
<td>11–20</td>
<td>136 (21.5)</td>
<td>4 (8.7)</td>
</tr>
<tr>
<td>21–30</td>
<td>162 (25.6)</td>
<td>12 (26.1)</td>
</tr>
<tr>
<td>31–40</td>
<td>142 (22.5)</td>
<td>12 (26.1)</td>
</tr>
<tr>
<td>41–50</td>
<td>80 (12.7)</td>
<td>7 (15.2)</td>
</tr>
<tr>
<td>51–60</td>
<td>77 (12.2)</td>
<td>9 (19.6)</td>
</tr>
<tr>
<td>Disease classification</td>
<td>422 (66.8)%</td>
<td>28 (60.9)%</td>
</tr>
<tr>
<td>Dengue fever</td>
<td>159 (22.2)%</td>
<td>14 (30.4)%</td>
</tr>
<tr>
<td>Dengue with complications</td>
<td>4 (0.6)%</td>
<td>2 (4.3)%</td>
</tr>
<tr>
<td>Not classified</td>
<td>47 (7.4)%</td>
<td>2 (4.3)%</td>
</tr>
</tbody>
</table>

Values shown in parenthesis are percentage; five records with missing data on age.

a DEN-1, dengue hemorrhagic fever.
with four sero-conversion in paired samples. Anti-dengue IgM/IgG antibodies were positive for 81.2% of the samples indicating cumulative dengue virus exposure.

DEN 1 belonged to RSS-PCR subtype C, and DEN 3 strains analyzed matched to subtype C. Both serotypes corresponded to the circulating strains in the country (De Simone et al., 2004). DEN 2 strains had similar profiles but could not be matched to known patterns.

4. Discussion

In Central Brazil, the city of Goiania evolved from high DEN 1 circulation in 2002 to a high circulation of DEN 3 in 2003. Despite the co-circulation of three different serotypes in recent years, DHF and DF cases are still reported mainly in adults, which characterize a different pattern from Asian countries and some endemic American countries (Guzman and Kouri, 2003; Siqueira et al., 2005). The reintroduction of DEN 3 in the Americas occurred in Nicaragua and Panama in 1994, spreading to most American countries (Briseno-Garcia et al., 1996; Nogueira et al., 2001; Barbosa da Silva et al., 2002; Rigau-Perez et al., 2002). A study in Nicaragua showed that children already represent the major burden of dengue after 15 years of dengue virus circulation, and DEN 2 was the prevailing serotype (Hammond et al., 2005).

In our setting, the virological surveillance does not suggest a major role of infecting DEN 3 serotype in increasing disease severity since the ratio of dengue fever to dengue with complications/DHF remained constant. These findings are in concordance with data from Puerto Rico where there was no increase in disease severity associated with DEN 3 introduction in 1998 (Rigau-Perez et al., 2002). In contrast, the introduction of DEN 3 in the Rio de Janeiro State led to almost 2000 DHF cases and 91 fatalities in 2002 (Casali et al., 2004; Nogueira et al., 2005). The association between severity of disease and introduction of DEN 3 serotypes in both settings in Brazil should be interpreted with caution since primary and secondary dengue infection cannot be distinguished by the official Brazilian surveillance system. In our study more than 20% of laboratory-confirmed dengue cases were classified as dengue with complications showing the difficult to fulfill the DHF criteria. Previous studies have pointed out that the application of the WHO criteria for case ascertainment may underestimate the number of DHF and/or its severe clinical manifestations, especially among the adult population (Rigau-Perez and Bonilla, 1999; Balmaseda et al., 2005). Sequential viral dengue infections, virulence of different virus strains, previous immune status, genetic diversity of the population, age-related differences and co-morbidity are possible explanations for disease severity (Halstead, 1997; Hammond et al., 2005). Therefore, multiple and complex factors may contribute to dengue severity. Our results, although limited to a subset of the dengue cases and dengue serotypes, reflects the recent introduction of DEN 3 in Central Brazil.

In this study, the serotypes identification by viral isolation showed low yield. Viral isolation is considered a laborious and time-consuming methodology with well-known limitation for large scale monitoring (Guzman and Kouri, 2004; Shu and Huang, 2004). Nevertheless, it is the gold standard technique for virus detection (WHO, 1997). The RT-PCR technique enhanced the laboratory-confirmed cases in this investigation in concordance with previous publications showing that a combination of RT-PCR and virus isolation increases the laboratory diagnosis of dengue. The advantage of RT-PCR is that it allows a timely identification of dengue serotypes for diagnostic and surveillance purposes. The relatively simple RSS-PCR typing applied helped to characterize the common origin of dengue-1, according to the classification previously described in Brazil (Miagostovich et al., 2000; Miagostovich et al., 2002). The pattern detected in the dengue-3 strain belonged to subtype C, with a variation (Harris et al., 1999) also detected in samples from Brazil (Miagostovich et al., 2002) and Nicaragua (Balmaseda et al., 1999).

Our findings represent an early stage of DEN 3 transmission and that the impact of this serotype on disease severity may still not be noticeable. Continuous monitoring of circulating serotype enhanced with molecular techniques is critical for dengue surveillance not only to detect the introduction of a new serotype, but also to understand the transition on disease severity and the shift from age groups among different populations and regions.

Acknowledgments

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