

## ***Epidemiological study of Dengue infections in patients attended in a Public General Hospital, in Rio de Janeiro City, Brazil, during the outbreak of 2001-2002***

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### ***Abstract***

***From November of 2001 until April of 2002, a total of 1124 blood samples were collected from patients suspected of dengue infection attended in the Hospital Municipal Paulino Werneck (HMPW), Ilha do Governador, Rio de Janeiro, Brazil. The samples were used for anti-dengue IgM and IgG detection, virus isolation, RT-PCR and Real time PCR. From those samples tested, 13.1% (147/1124) were positive by IgM capture enzyme-linked immunosorbent assay. From these, 95 (64.6%) presented only IgM and 52 (35.4%) showed the presence of anti-dengue IgG. Considering the rate between IgM and IgG > 1.5 as an indicative of primary infection, from the 147 infections by the dengue virus, 102 (69%) cases were characterized as primary infection and 45 (31%) as secondary infection. Among the seropositive patients the proportion of females, young adults and individuals residing in Ilha do Governador was higher. The population most affected was white without predominance of a specific gender, age and location of residence. The most significant clinical manifestations observed were rash and itching and leukopenia, lymphocytosis and thrombocytopenia constituted the main hematological profile observed in the patients with dengue infection. The epidemic occurred during the hottest months of the year where elevated temperatures and high pluvial index are common. A higher seropositivity was observed in serum samples collected between the 4th and 8th day after the onset of the disease. The virus serotype virus responsible for the epidemic belongs to serotype 3 and Real time PCR demonstrated to be more sensitive than the conventional technique used to detect the infecting serotype.***

***Keywords: Dengue- Epidemiology -Rio de Janeiro, Brazil.***

### ***INTRODUCTION***

***Dengue fever is an acute, mosquito-transmitted viral disease caused by four distinct serotypes DENV-1, DENV-2, DENV-3 and DENV-4<sup>1,2</sup> and along with Yellow Fever virus (YFV), Tick-Borne Encephalitis (TBEV), Japanese Encephalitis (JE), West Nile (WNV), Murray Valley Encephalitis (MVEV), St. Louis Encephalitis (SLEV) and many other viruses, belong to the Flavivirus genus<sup>3</sup>. After World War II dengue started to spread and since the 80's it has been***

***emerging as the most important viral disease transmitted by arthropods<sup>2,4,5</sup>. In Brazil, the first epidemic occurred in 1981-1982, in Boa Vista - Roraima, and it was caused by serotypes 1 and 4<sup>6</sup>. Since 1986 where one the most important epidemics occurred in Rio de Janeiro caused by DENV-1, many epidemics have been occurring in several states in the country<sup>7</sup>. Several years later, DENV-2 was introduced in Rio de Janeiro causing an epidemic where DENV-1 and DENV-2 co-***

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circulated. However, the most severe epidemic occurred in 2001 when DENV-3 was introduced in the state<sup>8,9</sup>. More recently, in the year of 2008 a new epidemic of Dengue occurred in the Rio de Janeiro State. Between January and May there were 162.701 cases notified. From the total of notified cases 23.983 were classified as Classical Dengue and 1.032 as Hemorrhagic Dengue. The Serotype 2 was responsible for the majority of the confirmed cases.\* Dengue can be presented as two clinical forms the classic dengue fever (DF), which produces a self limited infection and the hemorrhagic dengue fever (DHF), characterized by a diffused capillary extravasation of plasma that can lead to a profound hypotension and shock<sup>2</sup>.

Differential diagnosis of dengue includes influenza, rubeola, malaria, measles, typhoid fever, leptospirosis, and a variety of other infections caused by arbovirus and can be performed by virus isolation, antigen detection, nucleic acid amplification and serological tests. The latter are the most widely used assays for dengue diagnosis. IgM-ELISA tests, for example, are very useful and reliable for diagnosis of current infections caused by DENV, since IgM usually increases in the first week after the onset of the disease, reaches a peak in the second week and can be detectable for 2 to 3 months after infection<sup>10</sup>. This study presents the epidemiological data obtained from a sample of patients attended in the emergency room and ambulatory facilities of a Public General Hospital in Rio de Janeiro city during the outbreak of 2001-2002.

## MATERIAL AND METHODS

### *Study design and population samples*

A cross-sectional descriptive study has been performed to evaluate: levels of IgM and IgG in individuals clinically suspected of dengue infection, virus serotype, time of higher seropositivity and other clinical epidemiological features. Serum from 1124 patients (512 males and 612 females) with suggestive symptoms of dengue infection, attended in Hospital Municipal Paulino Werneck (HMPW)

between November of 2001 and April of 2002, were studied. Five categories were considered to classify patients in racial group: white, black e others (yellow, mulatto and indigenous)<sup>11</sup>. The suggestive FD cases were defined as acute febrile disease followed by headache, retro orbital pain, myalgia, arthralgia, prostration, rash and hemorrhage in FHD suspected cases<sup>12</sup>. Some patients presented hematological profile constituted of leukopenia (leukocytes < 4.500/mm<sup>3</sup>), hemoconcentration (hematocrit with increase de 20%), thrombocytopenia (thrombocytes < 150.000/mm<sup>3</sup>) and lymphocytosis (lymphocytes > 48%)<sup>12,13</sup>. After written consent and approval by the Ethical Committee in Research from the Hospital Universitário Clementino Fraga Filho (HUCFF), of the Universidade Federal do Rio de Janeiro (UFRJ), patients have been interviewed for the epidemiological study.

### *Clinical specimens*

One unique blood sample has been obtained from each patient. The samples were collected by the technical staff from HMPW, and centrifuged (centrifuge CELM MOD LSII, series 2126) at 285 x g for 5 minutes. The sera were classified and stored at -20°C until use.

### *Serological methods*

· IgM capture enzyme-linked immunosorbent assay (IgM-ELISA)

IgM-ELISA was carried out in 1124 dengue suspected cases by using the MAC-ELISA Kit produced and kindly provided by the Institute of Technology in Immunobiologics of Oswaldo Cruz Foundation, Rio de Janeiro, Brazil (Biomanguinhos), and performed according to the manufacturer instructions

· IgG capture enzyme-linked immunosorbent assay (IgG-ELISA)

IgG-ELISA was carried out in the positive samples to anti-dengue IgM for the immune response characterization as primary or secondary, as described by Innis and others<sup>10</sup>, with some modification. For detection of anti-dengue IgG the plates of microtitulation were sensitized with 10

\* See <http://www.saude.rj.gov.br/>

*mg/mL specific antibodies for immunoglobulin G (anti-g sgm Chemicals Co. EUA) and the antigenic preparations and the other reagents from IgM-ELISA kit were used developed by Bioman-guinhos*

#### **Virus isolation**

*The positive serum samples for anti-dengue IgM were used for viral isolation by inoculation into monolayers of C6/36 Aedes albopictus cell line<sup>14</sup> cultivated in growth medium Leibovitz-15 (Sigma Chemical Company, St. Louis, USA). After 10 days of inoculation the cultures were tested by indirect fluorescent antibody test (IFAT) using serotype-specific monoclonal antibodies for DENV<sup>15</sup>.*

#### **Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR)**

*The RT-PCR was performed in anti-dengue IgM positive samples (n=70) collected until the 5<sup>th</sup> day after the onset of the disease<sup>16</sup>.*

#### **Real time PCR**

*Real Time PCR for detecting typing and quantification DENV was performed, as described by Houng and others<sup>17</sup> with modification, in 6 from the 70 positive samples for anti-dengue IgM selected until the 5<sup>th</sup> day after the onset of the disease.*

#### **Statistical analysis**

*The obtained data were analysed by using Epi Info version 6.0 software<sup>18</sup>. To variable categorical, Qui-square test was used. As significance, were considered value of  $p < 0.05$ <sup>19</sup>.*

## **RESULTS**

#### **Laboratorial findings**

*From the 1124 patients analysed, 147 (13.1%) presented titers of anti-dengue IgM. 66 (44.9%) were males and 81 (55.1%) were females. Furthermore, 95 (64.6%) from 147 were reactive only for IgM and 52 (35.4%) were reactive*

*for both anti-dengue IgM and IgG. In the 147 positive cases, the infecting serotype could not be detected by viral isolation. The RT-PCR was performed in 70 acute sera from the 147 positive IgM cases selected until the 5<sup>th</sup> day after the onset of the disease. However, no infecting serotype was determined in the samples analysed. Real Time PCR was performed in 6 out of the 70 positive samples and identified the DENV-3 in 33% (2/6) of the cases with a number of particles up to  $10^2$  PFU/ml. A higher seropositivity was observed in serum samples collected between the 4<sup>th</sup> and 8<sup>th</sup> day after the onset of the disease (TABLE 1). Considering the rate between IgM and IgG  $> 1.5$  an indicative of primary infection in this study, from 147 infections by the dengue virus 102 (69%) were primary infections and 45 (31%) were secondary infections (FIGURE 1).*

#### **Population profile**

*In this study, among the seropositive patients, the proportion of females, young adults and individual residing in Ilha do Governador was higher. However, these variables were not statistically significant; the population most affected was white (TABLE 2).*

#### **Clinical findings**

*The most significant clinical manifestations observed were rash and itching and leukopenia, lymphocytosis and thrombocytopenia constituted the main hematological profile observed in the patients with dengue infection (TABLE 3). The highest number of cases was observed in February of 2002, however these data were not statistically significant (TABLE 1).*

## **DISCUSSION**

*In Brazil, some serological inquiries have been performed aiming to verify the infection rate by dengue viruses. Previous studies, in different samples, have reported that infection rates by the dengue viruses can vary from 25% to 56%<sup>20</sup>. In 1999, Araújo and others<sup>21</sup> detected anti-dengue IgM in 26.6% of the cases*

**studied using serum samples collected from dengue suspected cases from Pará, Brazil. In our study, from 1124 patients clinically suspected of dengue infection, only 13.1% of the cases were confirmed serologically, probably due to the sampling collection performed in the acute phase (< 72 h) in 70% of the cases<sup>21,22</sup>. The fact that a higher seropositivity was observed in serum samples collected between the 4th and 8th days after the onset of the disease supports this hypothesis. The results of negative IgM do not exclude the diagnosis of dengue. Furthermore, some of the patients could be presenting secondary**

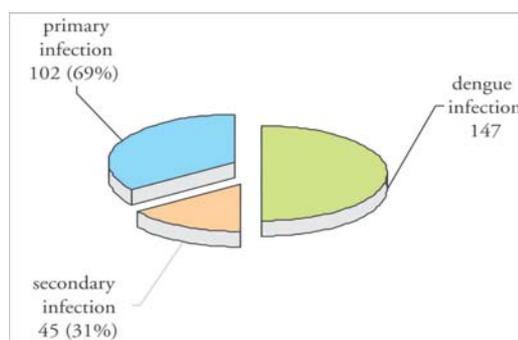


Figure 1 - Immune response in dengue positive patients (n=147) assisted in the Hospital Municipal Paulino Werneck – RJ (2001-2002).

Table 1 - Seropositivity for dengue according to the days after the onset of disease and month of the disease incidence.

	Positive [n = 147]	Negative [n = 977]	*Dengue cases				
			Total [n = 1124]	<sup>a</sup> OR	<sup>b</sup> IC	<sup>c</sup> X <sup>2</sup>	<sup>d</sup> p
<sup>e</sup> Number of days after the onset of the disease							
00							
01	02 (1.4)	50 (5.1)	52 (4.6)	0.3	0.0, 1.0	4.09	0.04
02	03 (2.0)	270 (27.6)	273 (24.3)	0.1	0.0, 0.2	44.14	0.000
03	02 (1.4)	226 (23.1)	228 (20.3)	0.1	0.0, 0.2	36.12	0.000
04	11 (7.5)	187 (19.1)	198 (17.6)	0.3	0.2, 0.6	11.17	0.001
05	30 (20.4)	132 (13.5)	162 (14.4)	1.6	1.0, 2.6	4.38	0.04
06	37 (25.1)	47 (4.8)	84 (7.4)	6.7	4.0, 10.9	73.68	0.000
07	28 (19.0)	21 (2.2)	49 (4.3)	10.7	5.6, 20.4	83.50	0.000
08	13 (8.8)	13 (1.3)	26 (2.3)	7.2	3.0, 17.2	28.68	0.000
09	13 (8.8)	09 (0.9)	22 (2.0)	10.4	4.0, 28.1	37.76	0.000
10	00 (0.0)	07 (0.7)	07 (0.6)	0.0	0.0, 4.6	0.22	0.64
11	00 (0.0)	04 (0.4)	04 (0.4)	0.0	0.0, 10.11	0.60	0.97
12	02 (1.4)	01 (0.1)	03 (0.3)	13.5	0.7, 795.0	3.61	0.06
13	01 (0.7)	01 (0.1)	02 (0.2)	6.7	0.1, 525.2	0.25	0.62
14	02 (1.4)	03 (0.3)	05 (0.4)	4.5	0.4, 39.4	1.27	0.26
15	00 (0.0)	02 (0.2)	02 (0.2)	0.0	0.0, 35.47	0.25	0.62
16	00 (0.0)	02 (0.2)	02 (0.2)	0.0	0.0, 35.47	0.25	0.62
17	00 (0.0)	02 (0.2)	02 (0.2)	0.0	0.0, 35.47	0.25	0.62
21	01 (0.7)	00 (0.0)	01 (0.1)	ind	ind	1.20	0.27
28	01 (0.7)	00 (0.0)	01 (0.1)	ind	ind	1.20	0.27
	01 (0.7)	00 (0.0)	01 (0.1)	ind	ind	1.20	0.27
Month of the disease incidence							
Nov/01							
Dec/01	00 (0.0)	01 (0.1)	01 (0.1)	0.0	0.0, 259.2	1.20	0.87
Jan/02	01 (0.7)	01 (0.1)	02 (0.2)	6.7	0.1, 525.3	0.25	0.24
Fev/02	40 (27.2)	354 (36.2)	394 (35.0)	0.7	0.4, 1.0	4.18	0.04
Mar/02	60 (40.8)	329 (33.7)	389 (34.6)	1.4	0.9, 2.0	2.57	0.11
Apr/02	41 (27.9)	235 (24.1)	276 (24.6)	1.2	0.8, 1.8	0.82	0.36
	05 (3.4)	57 (5.8)	62 (5.5)	0.6	0.2, 1.4	1.02	0.31

Notes: <sup>a</sup>OR = Odds ratio; <sup>b</sup>IC = Interval of the reliable level of 95%; <sup>c</sup>X<sup>2</sup> = Qui - square test; <sup>d</sup>p = Significance probability (p < 0.05); <sup>e</sup> Number of day after the first clinical symptoms of disease, where were collected the serum samples; (%).

Table 2 - Seropositivity for dengue in 1124 patients assisted in Hospital Municipal Paulino Werneck - RJ (2001-2002) according to the demographic characteristics.

Demographic characteristics	*Dengue cases						
	Positive [n = 147]	Negative [n = 977]	Total [n = 1124]	<sup>a</sup> OR	<sup>b</sup> IC	<sup>c</sup> X <sup>2</sup>	<sup>d</sup> p
Gender							
Male	66 (44.9)	446 (45.6)	512 (45.6)	1.0	0.7, 1.4	0.03	0.86
Female	81 (55.1)	531 (54.4)	612 (54.4)	1.0	0.7, 1.5	0.03	0.86
Age group							
0 - 9	29 (19.7)	150 (15.3)	179 (15.9)	1.3	0.8, 2.1	1.51	0.22
10 - 19	24 (16.3)	210 (21.5)	234 (20.8)	0.7	0.4, 1.2	1.77	0.18
20 - 29	37 (25.2)	217 (22.2)	254 (22.6)	1.2	0.8, 1.8	0.48	0.49
30 - 39	31 (21.1)	188 (19.2)	219 (19.5)	1.1	0.7, 1.7	0.17	0.68
40 - 49	20 (13.6)	105 (10.8)	125 (11.1)	1.3	0.7, 2.2	0.79	0.37
50 - 59	01 (0.7)	66 (6.8)	67 (6.0)	0.1	0.0, 0.6	7.36	0.007
60 - 69	04 (2.7)	28 (2.9)	32 (2.9)	0.1	0.2, 2,8	0.03	0.87
≥ 70	01 (0.7)	13 (1.3)	14 (1.2)	0.5	0.0, 3.4	0.07	0.79
Racial group							
White	66 (44.9)	318 (32.6)	384 (34.2)	1.7	1.2, 2.4	8.66	0.00
Black	07 (4.8)	98 (10.0)	105 (9.3)	0.5	0.2, 1.0	3.59	0.06
Other race <sup>e</sup>	74 (50.3)	561 (57.4)	635 (56.5)	0.8	0.5, 1.1	2.33	0.12
Residence location							
Ilha do Governador	113 (76.9)	711 (72.8)	824 (73.3)	1.2	0.8, 1.9	0.90	0.34
Bonsucesso	26 (17.7)	204 (20.9)	230 (20.5)	0.8	0.5, 1.3	0.62	0.43
Other localities <sup>f</sup>	08 (5.4)	62 (6.3)	70 (6.2)	0.9	0.3, 1.8	0.06	0.81

Notes: <sup>a</sup>OR = Odds ratio; <sup>b</sup>IC = Interval to the reliable level of 95%; <sup>c</sup>X<sup>2</sup> = Qui - square test; <sup>d</sup>p = Significance probability (p < 0.05); e = other race: yellow, brown and indigenous; f = other localities: Penha, Nova Iguaçu, Ramos (2 cases), São Cristóvão, Ilha do Fundão, Belford Roxo and Cordovil; (%).

**infection, characterized for elevated titers of anti-dengue IgG antibodies, where the levels of IgM antibodies can be very low and therefore they are not detectable<sup>21,22</sup>. In this study we only collected one blood sample and seroconversion in the IgG antibodies titer was not researched. For this reason, we predict that some positive cases (seroconversion) may have passed undetected in our approach (negatives false results). As secondary infections are more likely to cause DHE, the characterization of the patient immune response is always important during dengue epidemics<sup>10</sup>. The determination of titers of hemagglutinin inhibition antibodies (HI)**

**has been used to classify the infection by dengue virus as primary or secondary infections however; the HI test is a laborious technique<sup>10,23,24,25</sup>. In this study, to classify the cases of dengue as primary or secondary infections, anti-dengue IgM and IgG antibodies were quantified in units, using capture enzyme-linked immunosorbent assay (ELISA), and the rate between them was calculated<sup>10</sup>. Through this methodology, 31% of dengue cases were correctly classified as secondary infection. This methodology can be an excellent option to classify the dengue infections as primary or secondary because besides utilizing only one serum sample and**

Table 3 - Clinical manifestations and hematological data in dengue positive cases from the Hospital Municipal Paulino Werneck – RJ (2001-2002).

	*Dengue cases						
	Positive [n = 147]	Negative [n = 977]	Total [n = 1124]	<sup>a</sup> OR	<sup>b</sup> IC	<sup>c</sup> X <sup>2</sup>	<sup>d</sup> p
Clinical manifestations							
headache	92 (62.6)	833 (85.8)	925 (82.3)	0.3	0.2, 0.4	43.55	0.000
fever	74 (50.3)	815 (83.4)	889 (79.1)	0.2	0.1, 0.3	85.56	0.000
myalgia	90 (61.2)	796 (81.5)	886 (78.8)	0.4	0.2, 0.5	30.19	0.000
rash	69 (46.9)	106 (10.8)	175 (15.6)	7.3	4.9, 10.8	123.87	0.000
vomiting	51 (34.7)	372 (38.1)	423 (37.6)	0.9	0.6, 1.3	0.49	0.48
nausea	100 (68.0)	699 (71.5)	799 (71.1)	0.9	0.6, 1.3	0.61	0.43
abdominal pain	72 (49.0)	526 (53.8)	598 (53.2)	0.8	0.6, 1.2	1.02	0.31
cough	22 (15.0)	107 (10.9)	129 (11.5)	1.4	0.8, 2.4	1.65	0.20
diarrhea	38 (25.8)	218 (22.3)	256 (22.8)	1.2	0.8, 1.8	0.72	0.40
itching	67 (45.6)	122 (12.5)	189 (16.8)	5.9	3.9, 8.7	97.68	0.000
hemorrhage	47 (32.0)	99 (67.3)	146 (13.0)	4.2	2.7, 6.3	52.01	0.000
prostration	112 (76.2)	781 (79.9)	893 (79.4)	0.8	0.5, 1.2	0.88	0.35
anorexia	110 (74.8)	664 (68.0)	774 (68.9)	1.4	0.9, 2.1	2.50	0.11
arthralgia	52 (35.4)	418 (42.8)	470 (41.8)	0.7	0.5, 1.1	2.59	0.11
retro orbital pain	51 (34.7)	406 (41.6)	457 (40.7)	0.7	0.5, 1.1	2.22	0.14
Hematological data							
leukopenia	111 (75.5)	542 (55.5)	653 (58.1)	2.5	1.6, 3.8	20.25	0.000
hemoconcentration	05 (3.4)	48 (4.9)	53 (4.7)	0.7	0.2, 1.7	0.36	0.55
thrombocytopenia	36 (24.5)	47 (4.8)	83 (7.4)	6.4	3.8, 10.6	69.51	0.000
lymphocytosis	14 (9.5)	21 (2.1)	35 (3.1)	4.8	2.2, 10.1	20.65	0.000

Notes: <sup>a</sup>OR = Odds ratio; <sup>b</sup>IC = Interval to the reliable level of 95%; <sup>c</sup>X<sup>2</sup> = Qui - square test; <sup>d</sup>p = Significance probability (p < 0.05); (%).

*there is no need for titration or any previous treatment of serum samples, the capture ELISA possesses an excellent sensibility and specificity*<sup>25</sup>.

*The number of secondary infection cases observed in the study can be directly related with the high endemic of dengue in Rio de Janeiro State as well as co-circulation of serotypes 1, 2, and 3<sup>7,8,9</sup>. These secondary infections did not show significant statistical association with the presence of thrombocytopenia and hemorrhagic manifestations. It demonstrates that preexistence of a heterotypic humoral immunologic answer not always is associated to the disease severity<sup>26,27,28</sup>.*

*Although the detection of anti-dengue IgM was higher between 20 to 29 years (25.2%) the age did not correlate statistically with the dengue cases. The higher detection of anti-dengue IgM in*

*adults can result from the cohabitation and permanence of these people, with the Ae. aegypti, in closed environments<sup>21,29,30</sup>. This can also be one of the explanations for the higher number of cases between females of this study, since the Ae. aegypti has intradomicile habits most of the time, and the time of permanence of these women in the residences causes consequently a larger exposure to the vector<sup>21,29,30,31,32</sup>.*

*In 1981, Guzman and others<sup>33</sup>, performing serologic study in Cuba, during an epidemic of DENV-2, verified higher seropositivity for dengue in white people. The same fact also occurred in other epidemic study in Cuba<sup>34</sup>. As well as in the referred works, our data also demonstrated that the white individual presents a higher predisposition to dengue<sup>35</sup>.*

*Hemorrhagic manifestations do not occur only in DHF. It can also be observed in classic cases of dengue, although with a lower frequency and severity* <sup>36</sup>. In this study the absence of hemorrhagic manifestations showed significant statistical association with the seropositivity for dengue (TABLE 3).

Our results demonstrated a statistically significant correlation between the presence of rash and itching with the infection dengue virus. During an epidemic of dengue in Guadeloupe in 1997, Desruelles and others <sup>37</sup>, evaluating the dermatologic alterations present in patients with confirmed diagnosis of dengue, verified that rash was the main dermatologic alteration observed in those cases. In Brazil, Uberlândia city, Minas Gerais State, between 1993 and 1998, Nunes-Araujo, Ferreira e Nishioka <sup>38</sup>, findings demonstrated the value of clinical picture in the diagnosis of dengue, showing that among the symptoms observed in the disease (fever, headache, retro orbital pain, arthralgia etc.), rash was the most common symptom observed. In another work performed in Brazil in 1994, in Bahia, Nogueira and others <sup>39</sup>, corroborating our findings, found an elevated percentage of rash and itching in patient with symptoms of dengue. This biological aspect is suitable with the presence of anti-dengue IgM and IgG, since immunologic basis demonstrate that IgM and IgG activate the complement system which recruits and stimulates inflammatory cells to liberate vasoactive mediators, promoting the rash and consequently itching <sup>40</sup>.

The clinical diagnosis of the dengue, particularly in isolated cases, is relatively difficult to be performed. This difficulty is due to the clinical similarity that there is between dengue symptoms and other viruses. In the present work our results demonstrate that the triad leukopenia, lymphocytosis and thrombocytopenia, correlated statistically with seropositivity for dengue. We suggest that when associated to the clinical picture this hematologic profile constitutes an important tool in the presumptive diagnosis of dengue <sup>41</sup>.

The fact that such cases occurred from November 2001 to April 2002 demonstrated, as described by other authors, the seasonality of the infections by the dengue virus that comprehends

particularly, hot months where elevated temperatures and high pluviometric index propiciate the proliferation and survival of the *Ae. aegypti* <sup>30,42</sup>.

A limiting factor in the detection of positive results, particularly through the Reverse Transcription followed by Polymerase Chain Reaction (RT-PCR), could be due the digestion of viral RNA by the action of RNAses presents in the serum, since the samples were, for some times, thawed for manipulation and processing <sup>43</sup>. In addition, studies have demonstrated that immunoglobulins can inhibit the action of the polymerase during the process of amplification <sup>44</sup>. The Real Time PCR, corroborating to data from other studies, demonstrated to be a highly sensitive technique <sup>17</sup>. In this study, this methodology was able to detect DENV-3 and quantify up to  $10^2$  pfu/mL, in two of the six positive IgM samples. Our data demonstrates that serotype 3 was responsible for about 94% of the dengue cases occurred in 2002 in the state of Rio de Janeiro <sup>45</sup>, when the samples were collected. However, we could not discard the presence of other serotypes in the samples analysed.

In summary, this is an epidemiologic study which demonstrates that the population of Ilha do Governador was the target of one of the largest dengue epidemic of Rio de Janeiro <sup>45</sup>. Among the seropositive patients the proportion of females and un adults was higher. The most affected population was white without any selectivity for gender, age and location of residence. The most significant clinical manifestations observed were rash and itching and leukopenia, lymphocytosis and thrombocytopenia constituted the main hematological profile observed in the patients with dengue infection. The epidemic occurred during the hottest months of the year where elevated temperatures and high pluvial index are common. A higher seropositivity was observed in serum samples collected between the 4th and 8th day after the onset of the disease. The main serotype responsible for the epidemic belongs to serotype 3 and Real time PCR demonstrated to be more sensitive than the conventional technique used to detect the infecting serotype.

## ***Estudo epidemiológico de infecções por Dengue em pacientes atendidos em um Hospital Público na cidade do Rio de Janeiro, Brasil, durante a epidemia de 2001-2002.***

### **Resumo**

Entre o período de Novembro 2001 a Abril de 2002, foram coletadas um total de 1124 amostras de sangue de pacientes com suspeita de infecção por dengue atendidos no Hospital Municipal Paulino Werneck (HMPW), Ilha do Governador, Rio de Janeiro, Brasil. As amostras foram utilizadas para detecção de anticorpos anti-Dengue (IgM e IgG), isolamento viral, RT-PCR e PCR em tempo real. Destas amostras, 13.1% (147/1124) apresentaram anticorpos anti-Dengue, 95 (64.6%) apresentaram somente IgM e 52 (35.4%) IgM e IgG. Considerando a relação entre IgM e IgG >1.5 um indicativo de infecção primária, das 147 infecções pelo vírus da Dengue, 102 (69%) foram caracterizadas como infecção primária e 45 (31%) como infecção secundária. Dentre os pacientes soropositivos, a proporção de mulheres, jovens adultos e pessoas residindo na Ilha do Governador se mostrou maior. A população mais infectada constituiu-se de pessoas brancas, sem predominância de sexo, idade e local de residência. As manifestações clínicas mais significativas, exantema e prurido, foram acompanhadas de leucopenia, linfocitose e trombocitopenia. A presente epidemia ocorreu durante os meses mais quentes do ano onde temperaturas elevadas e altos índices pluviométricos, são muito freqüentes. A maior soropositividade para Dengue foi observada em amostras coletadas entre o 4º e 8º dias após o início da doença e o principal vírus envolvido foi identificado como pertencente ao sorotipo 3 (DENV-3). Dentre as metodologias utilizadas para detectar e tipificar o sorotipo infectante a PCR em tempo Real, demonstrou ser a mais sensível de todas.

**Palavras-chave:** Dengue – Epidemiologia – Rio de Janeiro, Brasil.

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