ORIGINAL ARTICLE

Characterization of dengue cases confirmed using the database linkage technique: assessment of virus circulation in Belo Horizonte, Brazil, 2009-2014*

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Ana Carolina Lemos Rabelo¹ - ● orcid.org/0000-0002-5247-9266

Frederico Figueiredo Amâncio² - ● orcid.org/0000-0002-5732-7173

Carla Sayuri Fogaça Oiko³ - ● orcid.org/0000-0001-6556-7989

Marcela Lencine Ferraz⁴ - ● orcid.org/0000-0003-1560-5777

Mariângela Carneiro⁵ - ● orcid.org/0000-0002-9390-7714

Abstract

Objective: to describe dengue virus circulation in Belo Horizonte, Brazil, from 2009 to 2014. **Methods**: this is a series study of cases of dengue infected by different virus serotypes, identified by virus isolation or RT-PCR; database linkage was performed between the Notifiable Health Conditions Information System (SINAN), the Brazilian National Health System Hospital Information System (SIH/SUS) and the Laboratory Environment Manager (GAL). **Results**: 91.1% of the records were linked (n=775); among the cases (n=851), 60.4% (n=514) were confirmed as DENV-1, 22.1% (n=188) DENV-4, 9.8% (n=83) as DENV-2, and 7.7% (n=66) as DENV-3; DENV-2 accounted for a higher percentage of severe cases (4.5%). **Conclusion**: DENV-1 prevailed and circulated in all evaluated years.

Keywords: Dengue; Dengue Virus; Epidemiological Monitoring; Database.

Correspondence:

Ana Carolina Lemos Rabelo – Instituto Oswaldo Cruz, Instituto René Rachou, World Mosquito Program, Av. Augusto de Lima, No. 1715, Anexo 31, Barro Preto, Belo Horizonte, MG, Brazil. Postcode: 30190-002 E-mail: aclrabelo@gmail.com



¹Universidade Federal de Minas Gerais, Programa de Pós-Graduação em Ciências da Saúde, Belo Horizonte, MG, Brazil

²Fundação Hospitalar do Estado de Minas Gerais, Hospital João XXIII, Belo Horizonte, MG, Brazil

³Secretaria Municipal de Saúde de Belo Horizonte, Gerência de Zoonoses Norte, Belo Horizonte, MG, Brazil

⁴Secretaria de Estado de Saúde de Minas Gerais, Superintendência de Vigilância Epidemiológica, Belo Horizonte, MG, Brazil

⁵Universidade Federal de Minas Gerais, Departamento de Parasitologia, Belo Horizonte, MG, Brazil

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Introduction

Dengue is an acute febrile disease. Its clinical spectrum varies from asymptomatic and oligosymptomatic forms, to severe forms and even death. It is considered to be one of the most important arboviruses in the world. The dengue virus (DENV) is transmitted to humans by mosquitoes of the *Aedes sp.* genus and has four distinct serotypes: DENV-1, DENV-2, DENV-3 and DENV-4. All four serotypes have been in circulation in Brazil since 2010.

The occurrence of dengue has been recorded in Brazil since 1846, while the first epidemic was recorded in 1981-1982 (Boa Vista, Roraima). The first cases in Belo Horizonte were recorded in 1996. Introduction of the four serotypes happened progressively, and between 2009 and 2014 all the serotypes circulated in the municipality at some time. In Belo Horizonte, virus identification is done by the Ezequiel Dias Foundation (FUNED) — the Minas Gerais state reference laboratory —, using virus isolation and reverse transcription polymerase chain reaction (RT-PCR) techniques. The laboratory results are input to the Laboratory Environment Manager (GAL).

Dengue epidemics place a significant burden on health services and the economy. Controlling the disease and reducing the density of its vector continue to be challenges.

In Brazil, all suspected dengue cases should be recorded on the Notifiable Health Conditions Information System (SINAN).¹ Cases requiring hospitalization are recorded on the National Health System Hospital Information System (SIH/SUS).¹ These systems do not have a unique identification field so that it is not possible to directly identify records of the same case on the different databases.

The database linkage technique has been used in Public Health analyses to detect and exclude duplicated records, as well as to integrate and aggregate information from different systems.^{6,7} In this way it is possible to identify and retrieve information held on one system but missing or incomplete on another,⁸ thus building a single and more complete database.^{6,7,9}

Dengue epidemics place a significant burden on health services and the economy. Controlling the disease and reducing the density of its vector continue to be challenges. Severity of symptoms, as well as risk of epidemics, can depend on the serotype in circulation, so that it is important to assess and describe the dynamics of infections caused by DENV.

The objective of this study was to describe dengue virus circulation in Belo Horizonte between 2009 and 2014.

Methods

This is a dengue case series study.

Belo Horizonte is the capital of the state of Minas Gerais, located in the Southeast region of Brazil. In 2010, the municipality had a population of 2,375,151 inhabitants, ¹⁰ divided into nine administrative regions (Barreiro, Centro Sul, Leste, Nordeste, Noroeste, Norte, Oeste, Pampulha and Venda Nova) coinciding with the territories of the Regional Health Directorates. The Regional Health Directorates are subdivided into 152 Health Center catchment areas.

The study included all dengue cases with positive DENV samples, analyzed by means of RT-PCR and/or virus isolation, between 2009 and 2014, in Belo Horizonte.

In order to identify the serotype — by-PCR and/or virus isolation —, blood samples were collected up to the fifth day following onset of symptoms and were sent to FUNED.

Serology testing and Non Structural 1 (NS1) antigen rapid testing were also assessed by the study as laboratory diagnosis methods. These methods do not allow serotype identification and were assessed as variables of interest but were not used as inclusion criteria. Serology tests are performed after the 6th day following the onset of symptoms and NSI testing is done up until the 5th day. In Belo Horizonte, the NS1 rapid test is used for sample screening for virus isolation. Positive samples and 10% of negative samples are sent to FUNED, up to a maximum limit of 90 samples a month.

The SINAN, SIH/SUS and GAL databases were linked. The three databases analyzed referred to the period between January 1st 2009 and December 31st 2014, comprising total records as follows: 263,995 (SINAN), 4,215 (SIH/SUS) and 4,392 (GAL).

The databases were prepared manually beforehand with regard to field standardization and coding. The same codes were applied for the 'sex' fields and the same data format was applied. The 'race/skin color', 'methodology' and 'serotype' fields were transformed into numerical characters.

The databases were imported into the MySQL Database Manager System (version 5.7) using the Front 5.2 tool. The databases used had some identifiers in common and, at this stage, a key was created with the 'person's name', 'mother's name' and 'date of birth' variables.

Considering the size of the databases used, the accuracy of linking techniques⁸ and the use of manual revision to complement the strategy, the option was taken to use deterministic linkage (based on exact agreement).

The INNER JOIN keyword was used between the databases, in the sequence GAL ↔ SINAN and GAL ↔ SIH/SUS, to link the databases and identify common records. ¹¹ In the case of unpaired names, considering that there were missing or badly filled in fields, and in the attempt to pair once more, the integrity of the key was reduced by removing variables in the following order: (i) Year of birth; (ii) Month of birth; (iii) Day of birth; (iv) Mother's 3rd surname; (v) Person's 3rd surname; (vi) Mother's 2º surname; (vii) Person's 2nd surname; (viii) Mother's name; and (ix) Person's name.

Unlinked records were reviewed manually, by one of the researchers (ACLR), in an attempt to achieve pairing.

Virus monitoring data were retrieved from GAL; hospitalization data were retrieved from SIH/SUS; while the remaining data (sociodemographic, final classification laboratory diagnosis, case closure, case progression, clinical manifestations and other data) were retrieved from SINAN.

The variables studied and their categories were:

- a) sex (male; female);
- b) age range (in years: \leq 10; 11-20; 21-30; 31-40; 41-50; >50);
- c) race/skin color (white; black; brown; yellow);
- d) schooling (illiterate; incomplete/complete elementary education; incomplete/complete high school education; incomplete/complete higher education);
- e) serotype (DENV-1; DENV-2; DENV-3; DENV-4);
- f) virus identification technique (virus isolation; RT-PCR);
- g) final classification (classic dengue; dengue with complications; dengue hemorrhagic fever; dengue; dengue with warning signs; dismissed case);
- h) case closure criterion (laboratory; clinical/epidemiological);
- i) case progression (cure; death from dengue; death from other causes);
- j) serology and rapid NSI test result (reactive; non-reactive; inconclusive);
- k) hospitalization (yes; no);

l) time between symptom onset and hospitalization; and
 m) length of hospital stay in days (mean; standard deviation [SD]; median).

Summary measures (mean; median; SD) were used for quantitative variables. Pearson's chi-square test (X²) was used to check for association of the 'sex' and 'age range' variables with the virus serotypes, taking a 5% significance level.

During the study period there was a change in the classification of dengue cases used by the Ministry of Health. To overcome this problem, the study population was divided into two periods: 2009-2013, cases were classified as classic dengue (CD), dengue with complications (DCC), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS); 2014 onwards, cases were classified as dengue (D), dengue with warning signs (DwWS) and severe dengue (SD). In order to analyze the dynamics or virus circulation in Belo Horizonte, CD and D cases were grouped together as 'classic dengue' and DCC, DHF, DSS, DwWS and SD cases were grouped together as 'non-classic dengue'.

The databases were stored and analyzed on SPSS[™] version 20.

The Study was approved by the Federal University of Minas Gerais Research Ethics Committee (CEP/UFMG): Certification of Submission for Ethical Appraisal (CAAE) No. 62191116.1.0000.5149, on December 7th 2016. Nominal data were used only for database linkage and participant privacy was preserved.

Results

Between 2009 and 2014, 250,632 suspected dengue cases in Belo Horizonte were notified on SINAN. 163,947 cases were confirmed and the highest notifications were found in 2010 (51,755) and 2013 (96,121). In the same period, 4,376 blood samples were sent to FUNED for DENV identification.

A total of 851 cases confirmed by virus isolation and/or RT-PCR were included in the study, 796 (93.5%) of which were recorded on SINAN. All 47 cases recorded on SIH/SUS were also recorded on SINAN. The case selection flowchart is shown in Figure 1. Table 1 shows the main sociodemographic characteristics of these cases. It is noteworthy that 52.6% of cases were female; 5.8% of cases were 10 years old or under, 72.2% were between 11 and 50 years old and 19.6% were over 50 years old, so that mean case age was 34.8 years (standard deviation: 17.8 years).

The DENV-1 serotype was identified in 514 cases (60.4%), DENV-4 in 188 (22.1%), DENV-2 in 83 (9.8%) and DENV-3 in 66 (7.7%). Of the total positive samples, 67.2% were confirmed by virus isolation. In 2009 and 2010, three serotypes circulated in Belo Horizonte: DENV-1, DENV-2 and DENV-3. In 2011, only the DENV-1 serotype was isolated. In the period 2012-2014, the

DENV-1 and DENV-4 serotypes were isolated (Figure 2). In 2010 and 2013, which were epidemic years, circulation of three and two serotypes was identified, respectively.

Among cases identified as having the DENV-1 and DENV-3 serotypes, the most frequent age range was 11-30 years (DENV-1, 44.9%; DENV-3, 43.8%). DENV-2 was identified more in people aged 11-30 (41;5%),

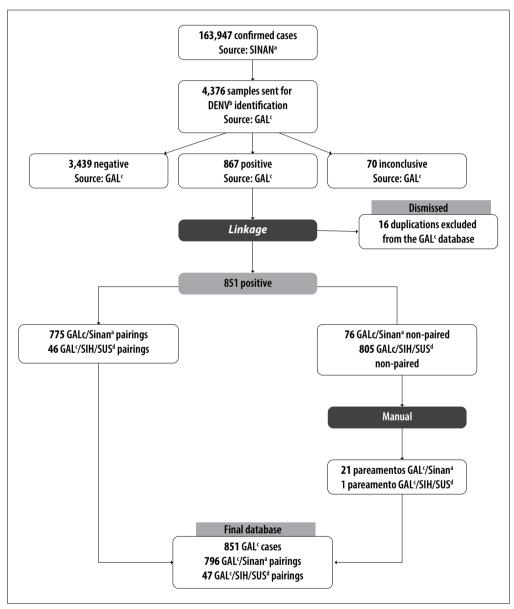


Figure 1 – Database linkage, Belo Horizonte, Minas Gerais, 2009-2014

a) SINAN: Notifiable Health Conditions Information System.

b) DENV: dengue virus.

c) GAL: Laboratory Environment Manager.

d) SIH/SUS: Brazilian National Health System Hospital Information System.

Table 1 – Sociodemographic characteristics of dengue cases confirmed by virus isolation and RT-PCR, a Belo Horizonte, Minas Gerais, 2009-2014

Characteristic	n = 851	%	
Sex			
Female	448	52.6	
Male	403	47.4	
Age (continuous variable)			
Mean \pm standard deviation	34.8 ± 17.8		
Median (interquartile ranges)	31.6 (21	.6;47.1)	
Age (in years – categorical variable)			
≤10	49	5.8	
11-20	155	18.2	
21-30	197	23.1	
31-40	151	17.7	
41-50	112	13.2	
>50	167	19.6	
Unknown/blank	20	2.4	
Race/skin color			
White	214	25.2	
Black	52	6.1	
Brown	190	22.3	
Yellow	6	0.7	
Unknown/blank	389	45.7	
Schooling			
Illiterate	80	9.4	
Incomplete elementary education	113	13.3	
Complete elementary education	45	5.3	
Incomplete high school education	.5 51	6.0	
Complete high school education	103	12.2	
Incomplete higher education	22	2.6	
Complete higher education	24	2.8	
Unknown/blank	413	48.5	
Virus monitoring — serotype	113	10.5	
DENV-1b	514	60.4	
DENV-2b	83	9.8	
DENV-3b	66	7.7	
DENV-4b	188	22.1	
Virus identification technique	100	22.1	
Virus isolation	572	67.2	
RT-PCRa	170	20.0	
Unknown/blank	109	12.8	
Final classification	107	12.0	
Classic dengue	590	69.3	
Dengue with complications	6	0.7	
Dengue hemorrhagic fever	3	0.4	
Dengue Dengue	128	15.0	
Dengue with warning signs	1	0.1	
Dismissed	49	5.8	
Unknown/blank	74	3.6 8.7	
Closure criterion	/4	0./	
Laboratory	403	01 A	
	693 85	81.4	
Clinical/epidemiological		10.0	
Unknown/blank	73	8.6	

to be continue

continuation

Table 1 – Sociodemographic characteristics of dengue cases confirmed by virus isolation and RT-PCR, Belo Horizonte, Minas Gerais, 2009-2014

Characteristic	n = 851	%
Case progression		
Cure	662	77.8
Death from dengue	6	0.7
Death from other causes	1	0.1
Unknown/blank	182	21.4
Serology		
Reactive	302	35.5
Non-reactive	32	3.8
Inconclusive	1	0.1
Unknown/blank	516	60.6
Rapid NS1 ^c test		
Reactive	369	43.4
Non-reactive	17	2.0
Unknown/blank	465	54.6
Hospitalization		
Yes	47	5.5
No	804	94.5

a) RT-PCR: reverse transcriptase reaction, followed by polymerase chain reaction.

while the DENV-4 serotype was identified more in the 31-50 age range (36.0%). There was no statistically significant difference in relation to 'sex' and 'age range' and circulating serotype (Table 2).

In 2009, the DENV-3 serotype was predominant in people under 10 years old, as well as those between 21-30 and 41-50 years old. DENV-2 was predominant in the 11-20 and over 50 age groups. In 2010, DENV-1 was the serotype with the highest percentage identified in all age ranges. In 2011, virus serotype was only identified in people who were 11-50 years old and only DENV-1 was isolated. In 2012, the serotype identified in the 10 years or under age range was DENV-4, while in the 11 years and over age ranges the predominant serotype was DENV-1. In 2013, the DENV-1 serotype was predominant among those under 30 years old, while the DENV-4 serotype was predominant among those over 31 years old. In the last year assessed, 2014, the predominant serotype in all age ranges was DENV-1 (Figure 3).

Between 2009 and 2014, 718 cases (98.6%) were classified as classic dengue and 10 (1.4%) as dengue with complications, dengue hemorrhagic fever and dengue with warning signs, grouped together as non-classic dengue (Table 2). With regard to other laboratory diagnosis methods, 335 (39.4%) cases also had dengue serology tests (Table 1), whereby 302 (35.5%) had a

reactive result, 32 (3.8%) were non-reactive and one (0.1%) was inconclusive. The rapid NS1 test was also performed for 386 cases and for 369 (43.4%) of them the test was reactive.

DENV-4 was the serotype identified for the highest percentage of rapid NS1 test (10.6%) and serology test (12.1%) negative cases. Serotypes DENV-2 and DENV-3 were not assessed for NS1 because in the years in which they circulated in Belo Horizonte, the rapid test was not in use.

Among cases for whom virus isolation and/or RT-PCR was performed, nine (1.1%) were classified as severe cases. Seven of them had hemorrhagic manifestations, two had hematuria and one had gastrointestinal bleeding.

With regard to the proportion of severe cases (non-classic) and classic cases, the highest percentage of severe cases (non-classic) was identified as having serotype DENV-2 (6.0%); while the highest percentage of classic dengue cases was identified as having the DENV-4 serotype (98.8%).

Of the total cases in the study, 47 (5.5%) were hospitalized and three (6.4%) needed care in an intensive therapy unit (ITU). Of the total hospitalized cases, 61.7% (n=29) were female and 66.0% (n=18) were between 11 and 50 years old. Mean time elapsing between onset of symptoms and hospitalization was 5 days (SD \pm 5), while the median was 4 days (interquartile ranges [IQ]

b) Dengue virus serotypes: 1, 2, 3 and 4.

c) NS1 antigen test.

Table 2 — Classification and characterization by sex and age of confirmed cases by dengue virus serotype, Belo Horizonte, Minas Gerais, 2009-2014

Classification and characteristics	Serotypes n (%)			T-4-1h		
	DENV-1ª	DENV-2ª	DENV-3ª	DENV-4ª	Total⁵	p-value ^c
Case classification						
Classic dengue	442 (99.1)	64 (95.5)	52 (98.1)	160 (98.8)	718 (98.6)	-
'Non-classic' dengue	4 (0.9)	3 (4.5)	1 (1.9)	2 (1.2)	10 (1.4)	_
Sex						
Female	266 (51.8)	46 (55.4)	30 (45.5)	106 (56.4)	448	0.416
Male	248 (48.2)	37 (44.6)	36 (54.5)	82 (43.6)	403	
Age range (in years)						
≤10	31 (6.2)	4 (4.9)	5 (7.8)	9 (4.8)	49	0.261
11-30	224 (44.9)	34 (41.5)	28 (43.8)	66 (35.5)		
31-50	148 (29.7)	24 (29.3)	24 (37.5)	67 (36.0)	263	
>50	96 (19.2)	20 (24.4)	7 (10.9)	44 (23.7)	167	

a) Dengue virus serotypes- 1,2,3 and 4.

c) Pearson's chi-square test.

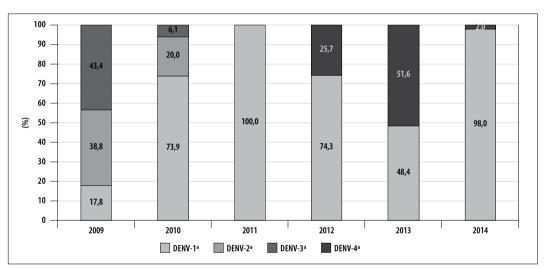


Figure 2 — Percentage of dengue virus serotypes by year of onset of symptoms, Belo Horizonte, Minas Gerais, 2009-2014

a) Dengue virus serotypes -1,2,3 and 4.

3;6). The mean number of hospital stay days was 3.8 (SD ± 2.3), while the median was 3.0 days (IQ 2.0;5.0).

Among hospitalized cases, 61.7% (n=29) were identified as having DENV-1, 25.5% (N=12) as having DENV-4, 10.6% (n=5) as having DENV-2 and 2.1% (n=1) as having DENV-3. With regard to ITU use, the highest percentage was found for cases with DENV-4: 66.7% (n=2).

With regard to case closure, 693 (89.1%) were closed according to the laboratory criterion and 85 according to

the clinical/epidemiological criterion (10.9%), despite the virus identification test result being positive. Of the total of cases for whom the 'case progression' field had been filled in, 662 (99.0%) progressed to cure, six (0.9%) died from dengue and one (0.1%) died from other causes. The highest absolute number of dengue deaths (n=4) occurred in people with the DENV-1 serotype. One case with the DENV-3 serotype died from dengue and another with the DENV-4 serotype died from other causes.

b) Totals variable due to missing information for this variable in the information source.

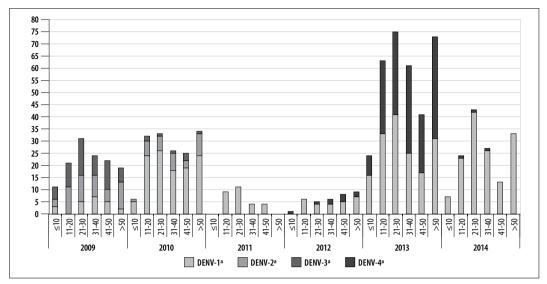


Figure 3 — Distribution of dengue virus serotypes by age range and year of onset of symptoms, Belo Horizonte, Minas Gerais, 2009-2014

a) Dengue virus serotypes - 1, 2, 3 and 4.

Discussion

Among dengue cases notified in Belo Horizonte between 2009 and 2014 and confirmed by virus isolation and/or RT-PCR, this study identified predominance of the DENV-1 serotype, which circulated throughout the entire period assessment, followed by DENV-4, DENV-2 and DENV-3.

The pattern of circulating dengue serotypes followed the national pattern, ¹² and the positivity of virus identification in Belo Horizonte was found to be below the national average. In Brazil as a whole, percentage positivity of virus identification in samples sent to reference laboratories was 36.8% in 2014, ¹³ and 39.2% in 2015. ¹⁴ In this study, among the samples tested, the highest percentage of seropositive results was 28.0% in 2013, an endemic year in which DENV-1 and DENV-4 circulated. A study conducted between 2010 and 2014 in Cuiabá, capital of the state of Mato Grosso, also found low percentage positivity and that 2012 was the year with the highest percentage of positive cases (23.1%). ¹⁵

In this study, females were predominant for all serotypes. This is in agreement with the findings of Martins et al. in relation to Salvador, capital of the state of Bahia, for the period 2007-2014. 16

By grouping cases together as classic cases and non-classic cases, we found that the percentage of cases classified as non-classic (DCC, DHF, DSS, DSCA and SD) is almost five times greater than that found on the municipal SINAN for the same period, when 0.3% of cases were non-classic. A study conducted in a public hospital in Rio de Janeiro, RJ, in 2008, during one of the biggest epidemics recorded in that municipality, found 6.7% non-classic cases.¹⁷

DENV-2 was the serotype with the highest percentage of severe cases in this study. Higher percentages of hospitalized cases were found for the DENV-4 and DENV-2 serotypes. These findings corroborate those of national study conducted by Burattini et al., ¹⁸ which showed a higher proportion of hospitalization related to DENV-2 serotype infection.

Distribution of hospitalizations by sex and age range was similar to cases reported in other places and periods. Between 1997 and 2002, in Belo Horizonte, a higher percentage of hospitalization of suspected dengue cases was reported among females (53.0%).¹⁹ The findings of our study corroborate those of that earlier study, according to which 60.4% of hospitalized cases were female. With regard to age range, between 1997 and 2002, in Belo Horizonte, the highest proportion of hospitalization due to dengue occurred in the 19-59 age group (65.0%),¹⁹ while in Goiânia, the capital of the state of Goiás, in the 2013 epidemic, the highest percentage of hospitalizations was among people who were 21-50 years old (45.8%) or older (27.1%) – the main age range hospitalized was the over 50s (37.5%).²⁰

In a study about the state of Minas Gerais, Campos²¹

found that on average hospitalization took place after four days following onset of symptoms in children and the elderly, and after five days following onset of symptoms in young people and adults. Our findings are similar to those of Campos, according to whom average number of hospitalization days (two days)²¹ was almost half that found in this study (3.8 days hospitalization).

It is noteworthy that in the years assessed, according to SIH/SUS data, overall percentage hospitalization due to dengue in Belo Horizonte was 2.2%, while our study found 5.5%. The fact of the rapid NS1 test, used for screening samples for virus identification, being available at emergency units and hospitals, usually dedicated to caring for more severe cases, may explain the higher percentage of hospitalization of virus isolation and/or RT-PCR positive cases.

The percentage of dengue deaths recorded on SINAN for the period and place studied was 0.02%. Our study found a percentage 4.5 times higher (0.9%), among those cases for which the 'case progression' variable had been filled in. This result may also be explained because of collection of samples for virus detection in severe cases.

With regard to laboratory diagnosis performed using other methods, of the total cases with positive virus isolation and/or RT-PCR for which NS1 was also performed, the rapid test result was positive for 95.5%. The highest percentage of negative NS1 tests corresponded to cases infected with the DENV-4 serotype. There are reports in the literature that differences in sensitivities between serotypes demonstrate that NS1 is less sensitive for detecting DENV-4 cases. ^{22,23}

In the period from 2009 to 2014, filling in the clinical manifestations field was not obligatory for classic dengue cases. It is supposed that the majority of classic cases had classical symptoms (fever starting suddenly, together with at least two of the following signs and symptoms: nausea/vomiting; exanthema; myalgia/arthralgia; headache/retro-orbital pain; petechiae/positive loop test; leukopenia)¹. Moreover, in epidemic years, there is great difficulty in filling in notification forms with a large number of variables.²⁴ As such, it was not possible to analyze associations between clinical manifestations and the different serotypes.

Among cases infected with the DENV-1 serotype, 1.2% had negative NS1, while among those infected with DENV-4, 10.6% had a negative NS1 test result.

This finding emphasizes the importance of sending a percentage of NS1 negative samples for virus isolation. The municipality of Belo Horizonte currently sends 10.0% of negative samples for virus isolation, this being a percentage similar to that found when assessing the DENV-4 serotype.

The data linkage technique enabled the use of complementary information from different databases, optimizing working hours and showing itself to be viable from the operational point of view. Deterministic linkage has greater specificity; however, this technique can "dismiss" records with potential for pairing, because of inconsistencies or typing errors.²⁵ In turn, probabilistic linkage increases the chance of pairing, but generates a larger number of pairs for manual reviewing, this being the main problem described in other papers.²⁶⁻²⁸ In view of its greater accuracy, use of deterministic linkage was defined for this study.

Standing out among the limitations of this study is the use of secondary data from databases with potential for losses, omission and/or incorrect records. The linkage technique allowed us to review the databases, assess information completeness and analyze the dynamics of virus circulation in Belo Horizonte. In addition, selection bias may occur in performing virus identification tests, given that the test used for sample screening is available in emergency units and hospitals that usually provide care to more severe cases. Moreover, the virus isolation technique is performed up until the fifth day after symptom onset, thus reducing the number of people with an adequate sample for this test, considering those who do not seek health care immediately. The way the sample is stored may also interfere with the possibility of performing this test.²⁹

Correct and quality completion of notification forms is essential for studies related to health and is beneficial for decision making. Lack of basic information on clinical manifestations on notification forms makes more in-depth analysis of the characteristics of infections caused by different DEN-V serotypes impossible. Notwithstanding, as mentioned above, in epidemic years, filling in lengthy notification forms is practically impossible.²⁴

The findings of this study emphasize the importance of good practices in relation to sample collection, storage and transportation, along with timely and adequate selection of cases in order to meet virus identification criteria. Implementation of these actions is feasible and they have positive impact on serotype identification and monitoring the dynamics of dengue.

We reiterate the importance of analyzing and describing characteristics of different DENV serotype infections. The recent history of dengue in Belo Horizonte and current epidemiological/entomological scenarios show greater susceptibility to the occurrence of new epidemics, highlighting that it is fundamental to gain more in-depth knowledge of the disease capable of aiding the formulation of more effective Public Health policies.

Authors' contributions

Amâncio FF, Ferraz ML, Carneiro M and Oiko CSF contributed to the concept and design of the article, analysis and interpretation of the results, drafting and reviewing the manuscript. All the authors have approved the final version and are responsible for all aspects thereof, including the guarantee of its accuracy and integrity.

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