ARTIGO ORIGINAL

Clinical, epidemiological, and laboratorial profile of arboviruses of suspected cases during Brazilian Zika virus emergence

Perfil clínico, epidemiológico y de laboratorio de casos sospechosos de arbovirus durante la emergencia del virus Zika en Brasil

Perfil clínico, epidemiológico e laboratorial de casos suspeitos de arboviroses durante a emergência do Zika vírus no Brasil

José Henrique Francisco Roma[1] ORCID 0000-0001-7990-6750
Rachel Cruz Alves[2] ORCID 0000-0002-5258-0292
Ludiele Souza Castro[3] ORCID 0000-0002-1717-0562
Márcio José Ferreira[3] ORCID 0000-0001-6475-9401
Claudinéia de Araújo[3] ORCID 0000-0002-3043-2731
Bruno Moreira Carneiro[3] ORCID 0000-0002-2355-7907
Renata Dezengrini Slhessarenko[2] ORCID 0000-0002-8084-8452
Juliana Helena Chavez Pavoni[3] ORCID 0000-0003-2568-9601
Mariângela Ribeiro Resende[1] ORCID 0000-0001-7923-0076

[1]Programa de Pós-Graduação em Clínica Médica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil.
[2]Programa de Pós-Graduação em Ciências da Saúde, Faculdade de Medicina Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brasil.
[3]Faculdade de Ciências da Saúde, Universidade Federal de Rondonópolis, Rondonópolis, Mato Grosso, Brasil.

Endereço: Universidade Federal de Rondonópolis, Faculdade de Ciências da Saúde. Avenida dos Estudantes 5.055, Cidade Universitária, Rondonópolis, MT 78735-910, Brasil.
E-mail: j262321@dac.unicamp.br

ABSTRACT

Background and Objectives: Arboviruses are public health issues worldwide related to severe human health impairment. These viruses cause similar acute febrile syndromes, challenging differential diagnosis and adequate notification. The work aimed to describe the epidemiological and laboratorial profile of arboviruses of suspected cases reported in southeastern Mato Grosso (MT) during the Zika virus emergence in Brazil. **Methods:** Epidemiological and clinical features of arboviruses were analyzed in a descriptive and retrospective study. Serum samples were assessed by real-time reverse transcriptase polymerase chain reaction for detection of Zika, Dengue and Chikungunya, and by immunoassay for detection of IgM and IgG against Zika. **Results:** Of 197 patients, 63% were female, eight were pregnant. Mean age was 32 years, with 44% between 19-59 years; 28% were white, 34% had educational instruction, and 58.9% were urban residents.

About 61% of cases occurred during rainy seasons, with 12.1% in three regions of the study area. Musculoskeletal disorders were the main complaints (22.8%), followed by fever (13.7%), rash and petechiae (12.6%), headache (11.7%), itching (10%), ophthalmological manifestations (6.1%) and gastrointestinal manifestations (5.6%). By means of molecular assays, 8.9% and 1.7% of Zika and Dengue were detected, respectively, in the samples tested. Serological assays for Zika showed 10.6% of samples positive for IgM and 55.8% for IgG. **Conclusions:** This study provides clinical, epidemiological, and laboratorial profile of Zika virus emergence in high-endemicity setting of dengue. The study relevance is understanding the introduction and concomitant occurrence of a new arbovirus disease.

Keywords: *Arboviruses. Epidemiology. Molecular Diagnostic. Serology.*

RESUMEN

Justificación y Objetivos: Los arbovirus son problemas de salud pública en todo el mundo relacionados con graves daños a la salud humana. Estos virus causan síndromes febriles agudos similares, lo que dificulta el diagnóstico diferencial y la notificación adecuada. El trabajo tuvo como objetivo describir el perfil epidemiológico y de laboratorio de los casos sospechosos de arbovirus notificados en el sudeste de Mato Grosso (MT) durante la emergencia del virus Zika en Brasil. **Métodos:** Se analizaron las características epidemiológicas y clínicas de los arbovirus en un estudio descriptivo y retrospectivo. Las muestras de suero se evaluaron mediante reacción en cadena de la polimerasa cuantitativa en tiempo real con transcripción inversa para la detección de de Zika, Dengue y Chikungunya. Anticuerpos IgM y IgG anti-Zika fueron detectados mediante inmunoensayo. **Resultados:** De 197 pacientes, 63% eran mujeres y ocho estaban embarazadas. La edad media fue de 32 años, con 44% entre 19-59 años; 28% eran blancos; 34% tenían instrucción educative; y 58,9% eran residentes urbanos. Alrededor del 61% de los casos ocurrieron durante las temporadas de lluvias, con 12,1% en tres regiones del área de estudio. Los trastornos musculoesqueléticos fueron las principales quejas (22,8%), seguidos de fiebre (13,7%), erupción y petequias (12,6%), dolor de cabeza (11,7%), picazón (10%), manifestaciones oftalmológicas (6,1%) y gastrointestinales (5,6%). Mediante pruebas moleculares se detectó un 8,9% y un 1,7% de Zika y Dengue, respectivamente, en las muestras analizadas. Las pruebas serológicas para Zika arrojaron un 10,6% de muestras positivas para IgM y un 55,8% para IgG. **Conclusiones:** Este estudio proporciona un perfil clínico, epidemiológico y de laboratorio de la aparición del virus Zika en uma región de alta endemicidad del dengue. La relevancia del estudio es comprender la introducción y aparición concomitante de una nueva enfermedad por arbovirus.

Palabras Clave: *Arbovirus. Epidemiología. Diagnóstico Molecular. Serología.*

RESUMO

Justificativa e Objetivos: Arboviroses impactam a saúde pública mundialmente, causando graves prejuízos à saúde humana. Os arbovírus causam síndromes febris agudas semelhantes, desafiando o diagnóstico diferencial e a notificação adequada. O objetivo desta pesquisa foi descrever o perfil epidemiológico e laboratorial dos casos suspeitos de arboviroses notificados no sudeste de Mato Grosso (MT) durante a primeira emergência

brasileira do Zika vírus. **Métodos:** as características clínicas e epidemiológicas das arboviroses foram analisadas de forma retrospectiva e descritiva. Amostras de soro foram avaliadas por reação em cadeia da polimerase em tempo real via transcriptase reversa para detecção de Zika, Dengue e Chikungunya, e por imunoensaio para detecção de IgM e IgG contra Zika. **Resultados:** Dos 197 pacientes, 63% eram do sexo feminino e oito estavam grávidas. A média de idade foi de 32 anos, dos quais 44% tinham 19 e 59 anos; 28% eram brancos; 34% eram alfabetizados; e 58,9% eram residentes urbanos. Cerca de 61% dos casos ocorreram durante os períodos chuvosos, sendo 12,1% em três regiões da área de estudo. Os distúrbios musculoesqueléticos foram as principais queixas (22,8%), seguidos de febre (13,7%), exantema e petéquias (12,6%), cefaleia (11,7%), prurido (10%), manifestações oftalmológicas (6,1%) e manifestações gastrointestinais (5,6%). Por meio de ensaios moleculares, foram detectados 8,9% e 1,7% de Zika e Dengue, respectivamente, nas amostras testadas. Os ensaios sorológicos para Zika mostraram 10,6% de amostras positivas para IgM e 55,8% para IgG. **Conclusão:** Este estudo fornece o perfil clínico, epidemiológico e laboratorial da emergência do Zika vírus em ambientes de alta endemicidade de dengue. A relevância do estudo é compreender a introdução e ocorrência concomitante de uma nova arbovirose.

Descritores: *Arboviroses. Epidemiologia. Diagnóstico Molecular. Sorologia.*

INTRODUCTION

Several required conditions are associated with the emergence of a new arboviral disease. Both introduction and dissemination must occur simultaneously to support the maintenance of complex cycles involving vectors, hosts, and human infection. In Brazil, the current climate changes associated with deforestation and socioeconomic conditions of the population represent critical aspects that are favorable to the emergence and reemergence of arboviruses transmitted by *Aedes* (*Stegomyia*) *aegypti* and *Aedes* (*Stegomyia*) *albopictus* mosquitoes.[1]

Although a significant number of Zika (ZIKV), Dengue (DENV), and Chikungunya (CHIKV) infections is asymptomatic, these viruses are responsible for moderate and severe syndromes. The acute febrile syndrome caused by DENV has been a significant public health challenge worldwide for decades, with a dramatic increase in cases in recent years, and it has been responsible for about 5,486 deaths until 39th epidemiological week of 2024 in Brazil.[2] Additionally, in recent years, two new emerging arboviruses were introduced in Brazil, ZIKV and CHIKV, both of which cause acute febrile syndromes and a severe impairment for human health. CHIKV is responsible for severe cases of polyarthralgia, and ZIKV, for congenital zika syndrome (CZS), leading to neuronal abnormalities, delayed neuro-psychomotor development in newborns, and Guillain-Barré syndrome (GBS) in adults, representing a public health emergency of

international concern according to the World Health Organization.[3-4]

The clinical symptoms associated with these arboviral infections are quite similar, especially in mild cases. Gastrointestinal, musculoskeletal, hematological, ophthalmological disorders, fever, itching, and headache are nonspecific symptoms of arboviral diseases, which hinder the differential clinical diagnosis and the correct notification of suspected cases. Therefore, effective notification associated with accurate diagnosis and the correct use of the Brazilian Health Information System are paramount and represent an impacting factor for public health.[5] The work aimed to describe the epidemiological and laboratorial profile of arboviruses of suspected cases reported in southeastern Mato Grosso (MT) during the emergence of ZIKV in Brazil.

METHODS

Study population

A retrospective and experimental study of suspected cases of arboviruses DENV, ZIKV and CHIKV fever reported in the municipality of Rondonópolis from August 2015 to August 2016 was carried out. During this period, Brazil experienced the emergence and dissemination of ZIKV.

Suspected cases of arboviruses assisted by the municipality's Primary Care Health Units were referred to the Central Laboratory of Rondonópolis, and clinical serum samples were obtained. The collected samples underwent molecular and serological analysis at the *Universidade Federal de Rondonópolis* Laboratory of Basic and Applied Virology. The study included patients up to five days after the onset of symptoms during the introduction of ZIKV in Brazil (2015-2016). Samples with more than five days of the onset of symptoms, insufficient volume for laboratorial analysis and inadequate storage conditions were excluded. Epidemiological data were obtained from July 2019 to November 2019 by notifiable forms, according to the Notifiable Diseases Information System (In Portuguese, *Sistema de Informação de Agravos de Notificação* - SINAN) of the Ministry of Health Brazil.

Demographic and clinical information analysis

The descriptive study was performed using sociodemographic and epidemiological data from 197 SINAN notifications; of these, 179 samples were included in laboratory analysis. Data were tabulated and analyzed using Microsoft Excel®

(Washington, USA). The spatial distribution of patients' household was achieved from the open-source Geographic Information System (QGIS v3.18) software.

Laboratory analysis

Molecular investigation was performed by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) in serum samples. The RNA was extracted and purified from 150μL of serum using silica columns (NucleoSpin® RNA Virus, Macherrey-Nagel, GER) according to the manufacturer's instructions. The one-step real-time ZDC Multiplex RT-PCR Assay® (BioRad, USA) was performed for RNA detection of DENV, ZIKV, and CHIKV viruses, following the manufacturer's instructions, on a CFX96 Touch™ Thermal Cycler (BioRad, USA).

Serological assessment was performed by enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies anti-ZIKV according to the manufacturer's instructions (Euroimmun AG, GER).

The interpretation of serological results was conducted carefully, considering the possibility of cross-reaction among arboviruses. In this regard, the molecular and serological diagnosis employed in this study present specific features, as shown on Table 1.[6]

Table 1. Comparative analysis of molecular and serological methods for arbovirus diagnosis

	Diagnostic assay	
	Molecular	**Serology**
Sampling	Acute phase - *until 5th day of symptoms*	Late phase - *after 6th day of symptoms*
Sample	Serum	Serum
Storage	-80ºC	-20ºC
Detection	Antigen - *Viral RNA*	Antibodies - *IgM/IgG*
Advantages	Early diagnosis	Lower cost
	High specificity and sensitivity	Easy technique execution
	Accurate and differential diagnosis	Tool for epidemiological surveillance
	Tool for molecular epidemiological surveillance - *genome sequencing*	Assessment and monitoring of the immune response
Disadvantages	Higher cost	Low specificity
	Complex technique execution	Cross-reaction with other arboviruses
		Inconclusive diagnosis

Legend: RNA – ribonucleic acid; IgM – immunoglobulin M; IgG – immunoglobulin G.

The study was conducted obeying the ethical aspects that regulate scientific research involving human, according to Resolution 466/12 of the Brazilian National

Health Council, and was approved by the *Universidade Federal de Mato Grosso* Research Ethics Committee, under CAAE 10767319.8.0000.8088.

RESULTS

This study included 197 patients with acute febrile syndrome and clinical suspicion of DENV, ZIKV, or CHIKV infections. According to the 197 patients' SINAN forms, 124 (63%) were female; of these, eight were pregnant, four in the first trimester and the other half in the third trimester of pregnancy. The mean age was 32 years, and the youngest patient was two months old, whereas the oldest was 70 years old. The most frequent age group was 19-59 years. Considering ethnic identity, 28% of patients self-declared as white, and three cases were reported as indigenous. The educational level was missing in 57% of notification forms. On the other hand, for 17 patients (8.6%), this variable was inapplicable according to age (Table 2).

Table 2: Sociodemographic features of arboviruses of suspected cases in Rondonópolis, Brazil, during the ZIKV Brazilian epidemic (2015–2016)

Variables		Frequency	
		n (197)	%
Gender			
	Female	124	63.0
	Male	69	35.0
	Ignored	4	2.0
Age range (years)			
	0–5	16	8.1
	5–19	14	7.1
	19–59	88	44.7
	59–70	10	5.1
	Ignored	69	35.0
Ethnicity/skin color			
	White	56	28.4
	Black	7	3.6
	Yellow	0	0
	Brown	38	19.3
	Indigenous	3	1.5
	Ignored	93	47.2
Educational level			
	Unlettered	1	0.5
	Incomplete ES	19	9.7
	Complete ES	4	2.0
	Incomplete HS	6	3.0

	Complete HS	14	7.1
	Incomplete UE	5	2.5
	Complete UE	19	9.7
	Unapplicable	17	8.6
	Ignored	112	56.9
Area of residence			
	Urban	116	58.9
	Countryside	4	2.0
	Peri-urban	1	0.5
	Ignored	76	38.6

Legend: ES: elementary school; HS: high school; UE: university education.

With respect to patients' residence areas, 114 (57.9%) reported living in urban areas and three patients lived in indigenous territory of Rondonópolis. Only four notifications presented patients belonging to other municipalities, which are included to the same health region (southeast), according to the division of Mato Grosso Regions of Health.[7]

In our study, the included cases were distributed in 79 (33%) neighborhoods in the municipality of Rondonópolis. Geoprocessing analysis showed a high density of notified cases in three urban neighborhoods of the city, in the central and north, as shown in Figure 1.
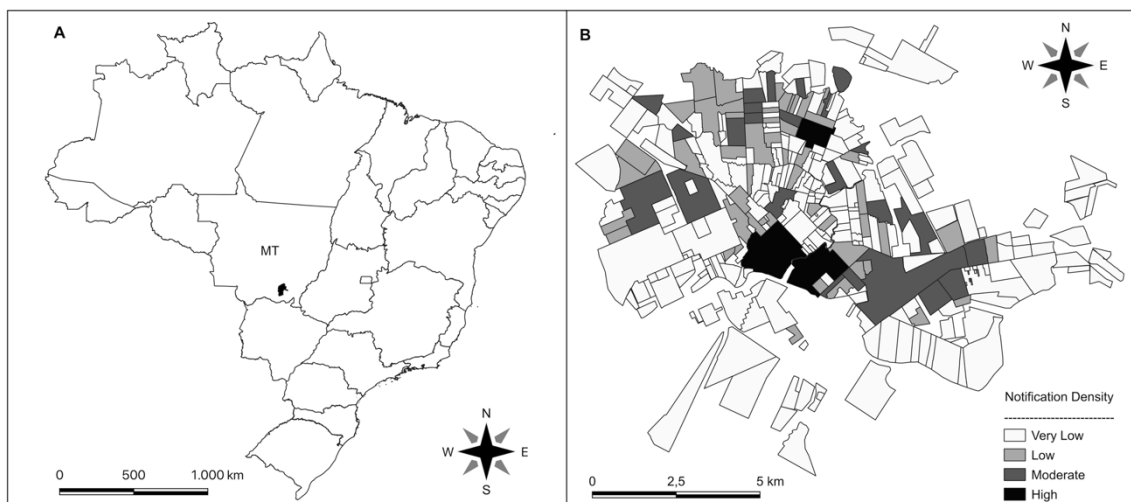

Figure 1. Geographic location of study area

Legend: maps of Brazil, state of Mato Grosso (MT) and the municipality of Rondonópolis (black) (**A**); spatial distribution of reported cases of ZIKV, DENV, and CHIKV by notification density among the urban neighborhoods in the municipality of Rondonópolis (**B**) during the ZIKV Brazilian epidemic (2015-2016).

According to clinical presentations data, only 46 (23.3%) forms had some

information. Musculoskeletal disorders such as arthralgia, joint swelling, and myalgia were reported for 45 (22.8%) patients, who presented at least one of these symptoms, representing the main complaints. Fever (13.7%), headache (11.7%), itching (10%), and symptoms related to hematological disorders, such as rash and petechiae (12.6%), were also notified. Other described symptoms were ophthalmological (6.1%) and gastrointestinal (5.6%) manifestations (Table 3).

Table 3: Clinical features of arboviruses of suspected cases in Rondonópolis, Brazil, during the ZIKV Brazilian epidemic (2015–2016)

Clinical signs/symptoms	Frequency	
	n (197)	%
Systemic		
Fever	27	13.7
Itching	20	10.1
Headache	23	11.7
Gastrointestinal		
Vomit		
Nausea	11	5.6
Abdominal pain		
Diarrhea		
Musculoskeletal		
Arthralgia		
Joint swelling	45	22.8
Myalgia		
Hematological		
Petechiae	25	12.6
Rash		
Ophthalmological		
Retro-orbital pain	12	6.1
Conjunctival hyperemia		

Experimental analyses were performed with a total of 179 serum samples (Table 4). Molecular assay revealed three (1.7%) patients infected with DENV. Additionally, 16 (8.9%) patients had positive results for ZIKV, and CHIKV RNA was not detected in any clinical serum samples. Therefore, a total of 19 patients tested positive in molecular assays performed by the study, of which 18 (94.7%) were women. Regarding the ZIKV serology, IgM was detected in 19 (10.6%) samples and IgG in 100 (55.8%).

Table 4: Molecular and serological investigation of serum samples of arboviruses of suspected cases in Rondonópolis, Brazil, during the ZIKV Brazilian epidemic (2015–2016)

Molecular detection Positive/n	Serological detection (ZIKV) Positive/n	
	IgM	IgG

DENV	3/179 (1.7%)	NA	NA
ZIKV	16/179 (8.9%)	19/179 (10.6%)	100/179 (55.8%)
CHIKV	ND	NA	NA

Legend: IgG: immunoglobulin G; IgM: immunoglobulin M; NA: not available; ND: not detected.

DISCUSSION

In this retrospective study, we assessed clinical, epidemiological, and laboratorial data of DENV, ZIKV, and CHIKV suspected cases in a Brazilian municipality located in southeastern MT, Midwest region of Brazil. Such geographic area is endemic for DENV, with the concomitant occurrence of ZIKV and CHIKV. Our study reveals unprecedented data for arboviruses in this geographic region during the introduction of ZIKV and CHIKV in Brazil, 2015-2016. Molecular arbovirus investigation was performed during the same period, in Cuiabá, the state capital of MT, which is a distant region.[8]

Rondonópolis has approximately 236,000 inhabitants, and is the reference point to 18 other cities belonging to the Southeast Health region. During the period 2015–16, the city reported 8,597 notifications of DENV, 535 of ZIKV, and 70 of CHIKV fever, with a high incidence of DENV and ZIKV fever during the same period. In 2015–16, ZIKV and CHIKV fever cases were reported in about half of all Brazilian municipalities, while probable DENV cases were reported in all of them. Therefore, Brazil and consequently MT registered an expressive number of DENV, ZIKV, and CHIKV notifications due to the introduction and expansion of the recently emerging arboviruses associated with the persistence and endemicity of DENV. And the increased number of notifications occurred mainly during rainy seasons, according to SINAN notifications.[7,9-13] Our data supported this observation, with the highest number of cases (61%) detected by molecular tests reported between December 2015 and April 2016, with poor impact in reducing cases through educational campaigns for vector control implemented by Brazilian health authorities.

Following the introduction of ZIKV and the beginning of the outbreak in 2015 in Brazil, accurate laboratorial diagnostics were not available for MT, as in most parts of the country. The first positive confirmed results were reported only in February 2016 by the epidemiological surveillance program of the Department of Health of Mato Grosso. On the other hand, ZIKV was detected in a human sample collected in the municipality

of Tapurah, northern MT, in August 2015.[8] The first positive cases in pregnant women were also confirmed in February 2016 from Primavera do Leste, a municipality belonging to the same health region of Rondonópolis, reinforcing viral circulation in this area.[13] However, the first suspected case of CZS in this region probably occurred in June 2015, which would confirm the circulation of ZIKV in MT in the middle of that year.[4] In the same way, the first laboratory-confirmed cases of CHIKV fever were reported only in March 2016.[7]

In this regard, adequate filling of notification forms and protocols available by Brazilian health authorities is essential for implementing epidemiological surveillance programs, especially considering the introduction of new arboviruses in DENV endemic areas, as observed in the CHIKV and ZIKV outbreak cases in recent years.[5] Therefore, the high number of variables missing in different fields of the notification forms assessed in our study highlight the need for proper filling of notification forms for suspected cases, as clinical diagnosis was essential for introducing these arboviruses in Brazil, mainly due to the lack of serological or molecular diagnoses.

As for socio-demographic analyses, Female patients accounted for almost 63% of the total cases in our study, and eight of them were pregnant. A similar rate is commonly observed in other surveys, probably because women are more likely to seek healthcare compared to men, at least for arbovirus investigation. This phenomenon could also be explained by a greater risk of exposure to mosquito bites because of women domestic costumes. Other relevant fact about these data can be closely related to the protocol established by Brazilian authorities, which focused on women, mainly during pregnancy, because of the occurrence of CZS, especially during 2015–16. This fact may have motivated females to search for more healthcare, thus resulting in a greater number of positive cases, as observed in our study.[11,14] A larger number of notifications was observed for patients in the age group of 19–59 years, which represents the economically more active population. Although professional occupation was not assessed in the study, this profile represents important insight for the local economy, as arboviruses affect patient productivity, with a critical impact on daily work routines. Similar patterns were shown in other profile epidemiological studies conducted in Brazil.[11,15]

It was observed that notifications were concentrated in urban areas with high population density and hence higher risk of infection. This fact reinforces and

corroborates the urban features of DENV, ZIKV, and CHIKV, transmitted by anthropophilic mosquitoes to humans, which act as amplification hosts for these arboviruses critical to public health.[16]

Although only three notifications were reported in indigenous territory, these rural areas are close to urban areas of Rondonópolis and may represent a potential risk zone for future outbreaks, depending on ecological conditions. Therefore, these suspected cases highlight the importance of consider indigenous territory in the municipality epidemiological surveillance programs.

Epidemiological features and climatic and ecological conditions, such as rainfall periods associated with high temperatures, may predict arboviral epidemics. These features, accompanied by disorderly urban growth, overpopulation, and precarious sanitary conditions, combined with inefficient vector control programs, result in more mosquito breeding grounds and provide conducive conditions for increased proliferation of infectious disease vectors, such as arboviruses. In this regard, low socioeconomic status and less education levels are both important for individuals and consequently familiar risk to contract these febrile syndromes.[17,18] Therefore, due to the accelerated and disordered urbanization process of the municipality of Rondonópolis, the notable social, economic, and environmental differences among all urban neighborhoods can influence the high incidence of these diseases.[16,19]

The three arboviral diseases investigated in this study are closely related and cause DENV-like clinical signs and symptoms. Moreover, about 80% of ZIKV cases are asymptomatic and may thus hinder differential clinical diagnosis, resulting in arbovirus misclassification. Therefore, the concomitant circulation of ZIKV, CHIKV, and other arboviruses in an area endemic to DENV, where laboratory tests are not always available for prompt confirmation, is a challenge for public health authorities and especially for health professionals, which in turn interfere with obtaining accurate diagnosis.[8,20-22]

In this scenario, it is important that clinical assessment is followed by laboratory diagnosis, especially molecular assays, which provide a specific diagnosis since the onset of symptoms. Serological assays may present the possibility of cross-reaction and false positive results for arboviruses with the synthesis of cross-reactive antibodies in patients previously exposed to some heterologous flaviviruses, often leading to inconclusive

serology diagnostics. Furthermore, serological assays do not determine which DENV serotype is indeed circulating, which is very relevant, mainly in epidemic situations.[11,6]

It is important to point out that molecular and serological assays may be performed at different stages of the arboviral disease. Molecular assays may only be feasible during viremia stages, approximately five days after symptom onset. On the other hand, serology can be applicable after antibody synthesis, which is detectable at the end of the viremia stage.[6] Thus, our results showed one positive DENV sample in the molecular assay with the presence of ZIKV IgM antibodies, probably due to cross-reactivity. In addition, 14 positive ZIKV IgM were negative by molecular test. Furthermore, IgM was not detected in other 12 from 16 ZIKV RT-qPCR positive samples, probably because antibody synthesis was not completed or under the ELISA detection limits. Therefore, only four samples reached concomitantly serological (IgM) and molecular positive diagnosis, which combined with the high number of IgG positive results reinforce the possibility of cross-reaction, since the virus had just introduced into the region, showing that serological diagnosis is neither adequate nor timely.

New viruses recently discovered in the Pantanal biome in MT are indicators of the possibility of future interactions and compromise of human health.[23] Interestingly, it was observed that only 2% (4) of patients were submitted to some laboratorial diagnostic test by public health services in Rondonópolis during clinical assessment, and all were negative for acute infection by serological analysis. However, about 10.6% (19) of 179 sampled patients had positive RNA detection in our molecular investigation (IgM). Similar rates have been observed in related surveys.[24]

In this context, the negative cases may have been probably caused by other pathogens not investigated during clinical follow-up. Considering the total number of reported notifications during the studied period, it is possible to infer that epidemiological and clinical criteria alone are insufficient for accurate diagnoses. Furthermore, accurate laboratory diagnosis may have implications for patient management and clinical evolution, such as effective treatment, and this highlights the importance of molecular assays for early detection and definitive confirmation of febrile syndrome etiologies.[25]

It is important to address the limitations of this study, which were related to the quality of data obtained from patients' SINAN forms, with high level of incomplete information. In addition to this, there is the possibility of other infectious etiologies since

the survey was designed only for DENV, ZIKV, and CHIKV molecular detection and for ZIKV serological investigation. Moreover, the samples were collected between 2015–16, and molecular analyses were performed only in 2019–20, indicating the possibility of viral RNA degradation.

In conclusion, our study provides singular local research concerning clinical and laboratorial assessment of suspected arboviruses infected patients during the first outbreak of ZIKV and CHIKV in Brazil, in a region that has shown a high incidence of DENV cases, representing a relevant contribution to understanding the concomitant occurrence of these acute febrile syndromes.

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