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Abstract: Chikungunya, Dengue, and Zika viruses are endemic to Brazil, with a high annual incidence rate. It is a serious public health issue and is responsible for outbreaks and epidemics worldwide. As COVID-19 emerged as a pandemic, the diagnosis of arboviruses was underestimated. Hence, this study aimed to characterize the sociodemographic and clinical aspects of arboviruses in the context of the COVID-19 pandemic in the state of Maranhão, Brazil. Sociodemographic and clinical data were obtained by applying questionnaires to 179 patients treated at health units in São José de Ribamar, Paço do Lumiar, Raposa, Santa Inês, and Vargem Grande municipalities. Serological and polymerase chain reaction analyses were performed do detect Chikungunya, Zika, Dengue, and COVID-19 infections. The Maranhão state registered 706 probable cases of Chikungunya from 2019 to 2020. In 2020, the cases were reduced by 74.8% compared to those reported in 2019. The test results were analyzed separately. Here, 46.3% of the Chikungunya virus test results were negative, and 36.8% were positive. For the Zika virus tests, most (79.9%) were positive, as well as the DENV1, DENV2, DENV3, and DENV4 tests (88.3%, 88.3%, 89.4%, and 65.9%, respectively). For the COVID-19 tests, of the 146 individuals tested, 52.7% were reactive when considering the anti-S test, and 17.8% were positive when considering the anti-N test. Fever and myalgia were the symptoms most frequently reported by patients. Our results emphasize the importance of continuous epidemiological surveillance of arboviruses in northeastern Brazil, and simultaneous testing for Chikungunya, Dengue, and Zika viruses among suspected patients.

Keywords: arboviruses; chikungunya; epidemiology; Covid-19

1. Introduction

Chikungunya virus (CHIKV) is an alphavirus of the *Togaviridae* family [1]. The CHIKV was first isolated by Ross (1952–1953) in Newala, Tanzania, where the main vector of the virus was the mosquito *Aedes aegypti*. Moreover, since then, outbreaks of CHIKV have become frequent in Africa, as seen in Kenya in 2004 and Cameroon Islands in 2005, crossing country boundaries, and reaching other territories, such as the islands Reunion, Seychelles, Mauritius, Madagascar, and Mayotte [2]. In 2006, in Reunion Island, there were more than 240,000 cases of Chikungunya and 250 deaths [3–5].

Dengue is a febrile infectious disease caused by an arbovirus of the *Flaviviridae* family and *Flavivirus* genus, transmitted mainly by the *A. aegypti* mosquito [6]. Dengue virus (DENV) has four different serotypes (DEN-1, DEN-2, DEN-3, and DEN-4), and occurs mainly in tropical and subtropical areas [7].

Zika virus (ZIKV) is an emerging arbovirus of the *Flaviviridae* family [8]. Infection also occurs through congenital transmission, sexual intercourse, and possibly through blood transfusion [9]. It is estimated that 80% of ZIKV infections are asymptomatic [10]. Although the common symptoms of ZIKV infection are mild, serious neurological complications, such as microcephaly in newborns and Guillain-Barré syndrome in adults, can occur in some cases.

According to data from the State Health Department, 370,645 COVID-19 cases were confirmed in Maranhão in December 2021, with approximately 10,000 deaths [11].

Considering the epidemiological importance of CHIKV infection and co-circulation of another arboviruses, this study aimed to investigate CHIKV infection in different municipalities of Maranhão State. This study investigated the prevalence of CHIKV infection and the co-circulation of DENV, ZIKV, and SARS-CoV-2.

2. Materials and Methods

Patients and serum samples

Peripheral venous blood was collected from 179 patients with suspected CHIKV disease from the Santa Inês (48 samples), Raposa (31 samples), Paço do Lumiar (24 samples), São José de Ribamar (27 samples), Vargem Grande (19 samples), and São Luís (30 samples) municipalities, as notified by the respective municipal health departments. All patients signed an informed consent form (ICF) to complete sociodemographic and clinical questionnaires related to the study. The inclusion criteria were based on the clinical manifestations observed in CHIKV disease, such as fever, and muscle and joint pain. Patients were ≥18 years. The exclusion criteria were related to clinical manifestations and laboratory tests positive for other diseases transmitted by the *A. aegypti* mosquito, such as dengue and Zika, and the use of pesticides.

Blood samples for the control group were collected from individuals who had never been diagnosed with chikungunya, with a total of 30 samples from the municipality of São Luís, MA.

The data were collected between December 2019 and February 2021. This study was approved by the Research Ethics Committee (CEP) of the Federal University of Maranhão (UFMA) by the opinion number 3.428.814.

Arboviruses tests

Enzyme-linked Immunosorbent Assay (ELISA) for detection of Zika, dengue, and chikungunya).

Serological evaluation was performed using ELISA to detect IgG anti-ZIKV, IgG anti-DENV, and IgG anti-CHIKV, based on a previous study [12]. High-affinity COSTAR 3590 plates (Corning, USA) were coated with 20 ng/well of ZIKV NS1, four DENV NS1 serotypes, and 25 ng/well of CHIKV E2 antigen (Meridian Life Science, USA), in carbonate-bicarbonate buffer pH 9.6 at 4° C overnight. The plates were blocked with 1% PBS-Tween buffer. Serum samples were diluted 1:400 and placed in duplicates on the plates. HRP-conjugated anti-human IgG antibodies (Promega, Madison, WI, USA) were added to wells at a 1:2000 dilution. The reaction was revealed by adding 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Sigma-Aldrich, USA) and hydrogen peroxide (Sigma-Aldrich, USA) to the plates, and was terminated by adding 5% sodium dodecyl sulfate (SDS) solution.

For each plate, three negative and four positive control samples were used to validate the test. The cutoff for the reaction was calculated from the corrected mean of the negative controls plus three times the standard deviation. The indeterminacy zone was defined as the range of reading values between 10% of the cutoff value upwards or downwards. The reaction was considered valid when at least three of the four positive controls presented valid positive results (above the indeterminacy zone). The readings were normalized for analysis by calculating the relative optical density, which was determined by the ratio of the sample optical density (OD) to the respective cutoff.

Chemiluminescent Microparticle Immunoassay (CMIA)

Serological evaluation for SARS-CoV-2 was performed using CMIA. The tests were performed using Abbott. In the anti-S test (dosage of IgG antibodies against SARS-CoV-2 subunit 1 [S1]), results are presented as AU/mL and the cutoff is 50.0 AU/mL for positive samples.

In the IgG anti-N test (research of IgG antibodies against SARS-CoV-2), results are presented as OD and the cutoff is 1.4 index for positive samples.

Viral controls and virus identification

Controls for DENV, ZIKV, and CHIKV available at the Molecular Biology of Infectious Diseases and Cancer Laboratory at the University of Rio Grande do Norte (were used. Viral controls were inoculated into tube cultures of *Aedes albopictus* clone C6/36 [13]. After inoculation, the cultures were incubated at 28° C and observed daily for 10–14 days. Positive cultures obtained using the viral isolation technique (which exhibited a cytopathic effect, ECP) were subsequently analyzed by RT-polymerase chain reaction (PCR) and qRT-PCR.

Viral RNA extraction and reverse transcription followed by PCR (conventional and real-time RT-PCR and qRT-PCR) were performed to detect DENV, ZIKV, and CHIKV.

Viral RNA was extracted from human serum samples using the QIAmp Viral Mini Kit (QIAGEN, Inc., Valencia, USA) according to the manufacturer's instructions. The extracted viral RNA was stored in -70° C freezer until use. Reverse transcription, followed by qRT-PCR using 7500 Fast Real-Time PCR System, was performed to detect ZIKV, as described by Faye et al. [14]. The methodology described by Lanciotti et al. [15] was used for detecting and typing the four serotypes of DENV simultaneously in a semi-nested procedure, generating amplified products (amplicons) of specific sizes (base pairs) for each DENV serotype. First, consensus primers (D1 and D2) were used for the four serotypes. In the semi-nested procedure, the specific primers TS1, TS2, TS3, and TS4 were used to detect DENV-1 to DENV-4. Agarose gel electrophoresis was performed to analyze the amplified products. To investigate CHIKV, the qRT-PCR protocol described by Lanciotti et al. was used [16].

This methodology required a fluorescence-labeled probe (VCHIK 6919P [Applied Biosystems]), a forward (VCHIKV 6856F) and a reverse primer (VCHIK6981R [both from Invitrogen]), as well as the sequence of interest. Aside from the primers, the TaqMan FAST Virus 1 Step Master Mix system (Applied Biosystems) was used to hybridize specific sequences of the genetic material of interest. The reagents were distributed in a 96-well plate, with a final volume of 15 μL of the mixture containing 5 μL of RNA extracted in the first step. For this experiment, ABI Prism 7500 Fast equipment (Applied Biosystems, Foster City, CA) was used following the thermocycling conditions [17].

Statistical analysis

Descriptive statistical analyses were performed using GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA). The data are presented in figures and tables. To verify the association between sociodemographic and clinical variables, $\chi 2$ test (Chi-square) was used, with p values ≤ 0.05 being considered significant.

To check the fit of the models, the R-squared ($R2$) was calculated. In addition, the performance of each model was evaluated using the area under the ROC curve (AUC) with 95% CIs. Finally, sensitivity and specificity were calculated.

Ethical Aspects

This study was approved by the Research Ethics Committee (CEP) of the Federal University of Maranhão (UFMA), by the opinion number 3.428.814. All the participants provided written informed consent.

For infants, the written Informed Consent Term was obtained with their relatives.

3. Results

This

Sociodemographic and clinical data

Samples from 179 patients with suspected arbovirus infections were collected from hospitals in São José de Ribamar, Paço do Lumiar, Raposa, Santa Inês, and Vargem Grande municipalities in the Maranhão State from 2019 to 2020.

The mean age was 38.5 years (standard deviation [SD] ± 17.4 years). From the 179 patients, 105 (58.7%) were female, 97 (54.2%) were mestizo, 64 (35.7%) completed high school, 107 (59.8%) were employed, and 79 (44.1%) had an income of half to one minimum wage (Table 1).

Age, dengue disease, origin (municipality), level of formal education, work status, and COVID-19 were found to be associated (Table 1).

Table 1. Sociodemographic characteristics of patients according to the patterns of infection, 2019–2021.

Variables	IgG CHIKV N=66(%)	IgG ZIKV N=143(%)	IgG DENV (serotypes 1–4) N=167(%)	IgG anti-S test N=77(%)	IgG anti-N test N=26(%)
Age (years)					
12–17	5 (7.6)	11 (7.7)	11 (6.6)*	7 (9.1)	4 (15.4)
18–39	32 (48.5)	63 (44.1)	78 (46.7)	32 (41.6)	9 (34.6)
40–59	20 (30.3)	49 (34.3)	54 (32.3)	30 (39.0)	10 (38.5)
>60	9 (13.6)	20 (14.0)	24 (14.4)	8 (10.4)	3 (11.5)
Sex					
Male	29 (43.9)	62 (43.4)	67 (40.1)	31 (40.3)	11 (42.3)
Female	37 (56.1)	81 (56.6)	100 (59/9)	46 (59.7)	15 (57.7)
Origin					
Paço do Lumiar	9 (13.6)	22 (15.4)	22 (13.2)	2 (2.6)*	1 (3.8)
Raposa	13 (19.7)	26 (18.2)	30 (18.0)	9 (11.7)	3 (11.5)
Santa Inês	15 (22.7)	32 (22.4)	43 (25.7)	32 (41.6)	12 (46.2)
São José de Ribamar	9 (13.6)	23 (16.1)	25 (15.0)	6 (7.8)	4 (15.4)
São Luís	15 (22.7)	25 (17.5)	30 (18.0)	17 (22.1)	3 (11.5)
Vargem Grande	5 (7.6)	15 (10.5)	17 (10.2)	11 (14.3)	3 (11.5)
Marital status					
Single	28 (42.4)	48 (33.6)	60 (35.9)	29 (37.7)	12 (46.2)
Married	33 (50.0)	84 (58.7)	96 (57.5)	45 (58.4)	13 (50.0)
Divorced	0 (0.0)	2 (1.4)	2 (1.2)	0 (0.0)	0 (0.0)
Separated	4 (6.1)	6 (4.2)	6 (3.6)	2 (2.6)	0 (0.0)
Widower	1 (1.5)	3 (2.1)	3 (1.8)	1 (1.3)	1 (3.8)
Race					
Caucasian	10 (15.2)	26 (18.2)	32 (19.2)	13 (16.9)	5 (19.2)
Black	15 (22.7)	37 (25.9)	45 (26.9)	18 (23.4)	5 (19.2)
Mestizo	41 (62.1)	80 (55.9)	90 (53.9)	46 (59.7)	16 (61.5)
Level of formal education					
Illiterate	3 (4.5)	7 (4.9)	8 (4.8)	3 (3.9)*	2 (7.7)
Incomplete primary education	7 (10.6)	15 (10.5)	15 (9.0)	4 (5.2)	1 (3.8)
Complete primary education	9 (13.6)	25 (17.5)	31 (18.6)	8 (10.4)	2 (7.7)
Incomplete high school	7 (10.6)	21 (14.7)	24 (14.4)	20 (26.0)	7 (26.9)
Complete high school	27 (40.9)	55 (38.5)	60 (35.9)	29 (37.7)	10 (38.5)

Incomplete higher education	5 (7.6)	10 (7.0)	15 (9.0)	5 (6.5)	2 (7.7)
Complete higher education	8 (12.1)	10 (7.0)	14 (8.4)	8 (10.4)	2 (7.7)
Work status					
Yes	38 (57.6)	89 (62.2)	102 (61.1)	55 (71.4)*	17 (65.4)
No	28 (42.4)	54 (37.8)	65 (38.9)	22 (28.6)	9 (34.6)
Income					
Until 1 minimum wage	23 (34.8)	61 (42,7)	73 (43,7)	29 (37,7)	11 (42,3)
1 to 1+1/2 minimum wage	23 (34.8)	50 (35)	57 (34,1)	29 (37,7)	9 (34,6)
1+1/2 to 2 minimum wage	20 (30.3)	32 (22,4)	37 (22,2)	19 (24,7)	6 (23,1)
Number of family members					
2	4 (6.1)	6 (4.2)	8 (4.8)	5 (6.5)	1 (3.8)
3	12 (18.2)	29 (20.3)	33 (19.8)	11 (14.3)	3 (11.5)
4	19 (28.8)	39 (27.3)	44 (26.3)	22 (28.6)	11 (42.3)
5	31 (47.0)	69 (48.3)	82 (49.1)	39 (50.6)	11 (42.3)

* Qui-square test. Statistical significance: p<0.05.

Tables 2 and 3 describe the odds ratios between the variables presenting a positive association, as described in Table 1.

People aged 18–39 years were 8.86 times more likely to test positive for dengue than were people aged 12–17 years; people aged 40–59 years were 8.18 times more likely to test positive for dengue than were people aged 12–17 years (Table 2).

Table 2. Odds Ratio (OR) between dengue and age.

	Positive		Negative		p-value	OR	CI 95%
	N	%	N	%			
Age (years)							
12–17	11	6.6%	5	41.7%	-	-	-
18–39	78	46.7%	4	33.3%	0.003	8.86	2.06–38.11
40–59	54	32.3%	3	25.0%	0.009	8.18	1.70–39.38
>60	24	14.4%	0	0.0%	0.998	-	-

CI: confidence interval.

People with incomplete primary education were 0.909 times less likely to have a positive anti-S test for COVID-19 than people with complete higher education were. People with complete elementary education are 0.867 times less likely to have positive anti-S test results for COVID-19 than were those with complete higher education. Moreover, people who did not work were 0.633 times less likely to have a positive anti-S test for COVID-19 than were people who worked (Table 3).

Table 3. Odds Ratio (OR) among anti-S tests for COVID-19 and sociodemographic variables.

Variables	Positive		Negative		p-value	OR	CI 95%
	N	%	N	%			
Origin							
Paço do Lumiar	2	2.6%	17	24.6%	-	-	-
Raposa	9	11.7%	12	17.4%	0.033	6.375	1.16–34.93
Santa Inês	32	41.6%	13	18.8%	<0.001	20.923	4.22–103.7
São José de Ribamar	6	7.8%	16	23.2%	0.192	3.187	0.56–18.16
São Luís	17	22.1%	4	5.8%	<0.001	36.125	4.82–224.2
Vargem Grande	11	14.3%	7	10.1%	0.004	13.357	2.33–76.48
Level of formal education							
Illiterate	3	3.9%	4	5.8%	0.128	0.188	0.02–1.62

Incomplete primary education	4	5.2%	11	15.9%	0.015	0.091	0.01–0.62
Complete primary education	8	10.4%	15	21.7%	0.026	0.133	0.02–0.78
Incomplete high school	20	26.0%	5	7.2%	1.000	1.000	0.16–6.26
Complete high school	29	37.7%	24	34.8%	0.153	0.302	0.06–1.56
Incomplete higher education	5	6.5%	8	11.6%	0.057	0.156	0.02–1.06
Complete higher education	8	10.4%	2	2.9%	-	-	-
Work status							
Yes	55	71.4%	33	47.8%	-	-	-
No	22	28.6%	36	52.2%	0.004	0.367	0.19–0.73

Clinical data analysis revealed that 176 (98.3%) patients had fever, 167 (93.3%) had myalgia, 29 (16.2%) had conjunctivitis, 86 (48.0%) had joint pain, 50 (27.9%) had pain when walking, 101 (56.4%) had skin eruptions, 92 (51.4%) had itching, 147 (82.6%) had nausea, 115 (64.2%) had vomiting, and 2 (1.1%) of the patients underwent the snare test. Fever was more prevalent among patients with Zika and dengue. Fever lasted longer in patients with dengue and COVID-19. Myalgia and skin eruptions were more frequent in patients with dengue. Conjunctivitis was more frequent in patients with Zika and dengue (Table 4).

Table 4. Clinical symptoms according to patterns of infection.

Variables	IgG CHIKV N=66(%)	IgG ZIKV N=143(%)	Dengue N=167(%)	Anti-S test N=77(%)	Anti-N test N=26(%)
Fever	65 (98.5)	140 (97.9)	164 (98.2)	77 (100.0)	26 (100.0)
Fever duration (days)					
0	3 (4.5)	9 (6.3)*	11 (6.6)	0 (0.0)*	0 (0)*
2	8 (12.1)	17 (11.9)	17 (10.2)	4 (5.2)	0 (0)
3	26 (39.4)	63 (44.1)	69 (41.3)	29 (37.7)	8 (30,8)
4	22 (33.3)	38 (26.6)	49 (29.3)	27 (35.1)	10 (38,5)
5	7 (10.6)	16 (11.2)	21 (12.6)	17 (22.1)	8 (30,8)
Myalgia	61 (92.4)	131 (91.6)	156 (93.4)	73 (94.8)	26 (100)
Conjunctivitis	11 (16.7)	25 (17.5)	27 (16.2)	10 (13.0)	2 (7,7)
Joint pain	36 (54.5)	74 (51.7)	83 (49.7)	34 (44.2)	9 (34,6)
Joint inflammation	10 (15.2)	25 (17.5)	31 (18.6)	13 (16.9)	5 (19,2)
Pain during walking	21 (31.8)	41 (28.7)	48 (28.7)	16 (20.8)*	7 (26,9)
Pain duration (days) and edema					
0	55 (83.3)	118 (82.5)	140 (83.8)	67 (87.0)	21 (80,8)
1	2 (3.0)	4 (2.8)	3 (1.8)	0 (0.0)	0 (0.0)
3	2 (3.0)	6 (4.2)	7 (4.2)	3 (3.9)	1 (3.8)
4	2 (3.0)	6 (4.2)	7 (4.2)	3 (3.9)	2 (7.7)
5	4 (6.1)	7 (4.9)	8 (4.8)	4 (5.2)	2 (7.7)
7	0 (0.0)	1 (0.7)	1 (0.6)	0 (0.0)	0 (0.0)
15	1 (1.5)	1 (0.7)	1 (0.6)	0 (0.0)	0 (0.0)
Skin eruptions	38 (57.6)	78 (54.5)	92 (55.1)	53 (68.8)*	18 (69.2)
Pruritus	35 (53.0)	72 (50.3)	83 (49.7)	46 (59.7)	20 (76.9)*
Nausea	53 (80.3)	114 (79.7)*	136 (81.4)	65 (84.4)	20 (76.9)
Vomit	43 (65.2)	92 (64.3)	107 (64.1)	53 (68.8)	17 (65.4)
Tie test	0 (0.0)	2 (1,.)	2 (1.2)	1 (1.3)	1 (3.8)

* Qui-square test. Statistical significance: p<0.05.

Regarding comorbidities, 16 (8.9%) patients had diabetes, 12 (6.70%) had hypertension, 9 (5.0%) had both diabetes and hypertension, and 142 (79.3%) had no comorbidities.

Anti- ZIKV, DENV and CHIKV antibodies

Test results were analyzed separately. Among CHIKV tests, 46.3% were negative, while 36.8% were positive. For the ZIKV tests, the majority (79.9%) was positive, as well as the majority of the DENV1, DENV2, DENV3, and DENV4 tests (88.3%, 88.3%, 89.4%, and 65.9%, respectively) (Table 5).

Table 5. Results of IgG anti-NS1 ZIKV, IgG anti-DENV (serotypes 1–4), and IgG anti-E2 CHIKV.

Variables	Positive	Negative	Indeterminate
IgG CHIKV	66 (36.8%)	83 (46.4%)	30 (16.8%)
IgG ZIKV	143 (79.9%)	20 (11.2%)	16 (8.9%)
DENV 1	158 (88.3%)	16 (8.9%)	05 (2.8%)
DENV 2	158 (88.3%)	16 (8.9%)	05 (2.8%)
DENV 3	160 (89.4%)	12 (6.7%)	07 (3.9%)
DENV 4	118 (65.9%)	42 (23.5%)	19 (10.6%)

COVID-19 analysis

From 146 patients, 7% were reactive in anti-S test, and 17.8% were positive in anti-N test.

The relation between COVID-19 and arboviruses was analyzed using the chi-square test, but no statistically significant relation was found.

Analysis of patients with positive qPCR for CHIKV

In addition to the serological testing, a viral identification survey was conducted on patient samples. The time of infection, as reported by the participants, was used as a criterion. We found that three had positive qPCR results for CHIKV infection.

Patient 1 was IgG-negative for CHIKV and other diseases except for DENV-4, which was indeterminate, leading to the assumption that the patient was in a transition state, starting to produce IgG antibodies. The patient was male, 50 years old, married, working, with an income between one and a half minimum wage. He had access to public water supply, and water storage in a water tank. He presented with five day-long fever, myalgia, joint pain, itching, nausea, and vomiting, without comorbidities.

Patient 2 had indeterminate IgG for CHIKV, and was positive for other diseases. The patient was female, 18 years old, single, not working, with an income between half and one minimum wage. She had access to public water supply, and water storage in a water tank. She presented with two day-long fever, myalgia, conjunctivitis, skin rashes, itching, nausea, vomiting, and diabetes.

Patient 3 was IgG-positive for CHIKV and other diseases, female, 49 years old, married, working, with an income between half and one minimum wage. She had access to public water supply, and water storage in a water tank. She presented 4 day-long fever, myalgia, joint pain, itching, nausea, vomiting, and no comorbidities.

Logistic regression

To check the fit of the models, the R-squared (R^2) was calculated. In addition, the performance of each model was evaluated using the area under the ROC curve (AUC) with 95% CIs. Finally, sensitivity and specificity were calculated (Table 6). For the Chikv IgG modeling, the R^2 statistic was very close to 0, this result can be explained by the fact that the predictor variables were included based on evidence that they are associated with this disease. The other models presented higher R^2 values, considering the inclusion of significant variables. The model for Dosage IGG stands out in relation to R^2, explaining 48.5% of the variance through the variables included in the model.

Observing the AUC of the models, it can be seen that it follows the R^2 measurement, since higher AUC values also had higher R^2 values. With the exception of models for IgG Chikv and IgG Zikv, all others had adequate AUC values (>0.7). It is worth mentioning that the model for Dosage IGG is the least parsimonious, that is, it contains many variables to explain the disease, in contrast, the others have only two or one explanatory variable.

Regarding the sensitivity and specificity of the models, the model for IgG Zikv showed the lowest sensitivity value, demonstrating that it can only predict 43% of the patients who actually had the disease. The model for researching IgG antibodies had the lowest specificity value, predicting 43% of non-ill patients correctly. The model for Dosage IGG obtained higher values of sensitivity and specificity, among all models.

Table 6. Diagnostic indices of multivariate models of factors associated with IgG ZIKV, Dengue, Anti-S and Anti-N test.

Arboviruses	R2	AUC	CI 95%	Sensitivity	Specificity
IgG Chikv[1]	0.020	0.557	0.470-0.643	0.714	0.645
IgG Zikv[2]	0.127	0.690	0.598-0.781	0.430	0.725
Dengue[3]	0.176	0.722	0.564-0.880	0.538	0.617
Anti-S test	0.485	0.848	0.786-0.909	0.758	0.763
Anti-N test	0,209	0,742	0,650-0,833	0,755	0,430

R2: percentage of response variable variation that is explained by the model; AUC: area under the curve; [1]: Adjusted model- Fever D., Pain when walking; [2]: Adjusted model - D. of fever, nausea; [3]: Adjusted model - Age; [4]: Adjusted model: Municipality, Education, Work, D. of fever, Pain when walking, skin eruptions; [5]: Adjusted model: D. of fever, itching.

Multivariate logistic regression analyzes were performed on each of the arboviruses (Table 7). Regression coefficients are presented as odds ratios (ORs) with 95% CIs (Table 7).

Table 7. Multivariate logistic regression of associated variables and arboviruses.

Variables	Regression coefficient (β) (95CI%)	OR (95CI%)	p-value
IgG Chikv			
Fever days			
0	-0.22 (-1.05 – 0.61)	0,80 (0.16 - 4.07)	0.790
2	0.68 (0.03 – 1.34)	1,98 (0.55 – 7.13)	0.297
3	0.38 (-0.13 – 0.89)	1,47 (0.54 – 3.99)	0.452
4	0.65 (0.12 – 1.18)	1,92 (0.68 – 5.42)	0.218
5	REF	-	-
Walking pain			
Yes	REF	-	-
No	-0.41 (-0.77 - -0.05)	0,66 (0.33 – 1.34)	0.253
IgG Zikv			
Fever days			
0	-	-	-
2	2.32 (1.21 – 3.44)	10,2 (1.14 – 90.85)	0.037
3	1.43 (0.87 – 1.99)	4,17 (1.4 – 12.42)	0.010
4	0.41 (-0.11 – 0.94)	1,51 (0.54 – 4.22)	0.434
5	REF	-	-
Nausea			
Yes	REF	-	-
No	1.1 (0.45 – 1.76)	3.02 (0.84 – 10.88)	0.092
Dengue			
Age (Years)			
12-17	REF	-	-
18-39	2.18 (1.44 – 2.93)	8.86 (2.06 – 38.11)	0.003
40-59	2.1 (1.3 – 2.9)	8.18 (1.7 – 39.38)	0.009
>60	-	-	-

Anti-S test			
Origin			
Paço do Lumiar	REF	-	-
Raposa	1.87 (0.87 – 2.86)	6.46 (0.92 – 45.28)	0.060
Santa Inês	2.73 (1.81 – 3.64)	15.26 (2.56 – 91.17)	0.003
São José de Ribamar	2.64 (1.57 – 3.72)	14.06 (1.71 – 115.52)	0.014
São Luís	4.01 (2.8 – 5.23)	55.37 (5.15 – 595.14)	0.001
Vargem grande	1.96 (0.88 – 3.03)	7.07 (0.87 – 57.71)	0.068
Schooling			
Illiterate	0.06 (-1.58 – 1.7)	1.06 (0.04 – 26.6)	0.970
Incomplete Elementary School	-0.33 (-1.76 – 1.11)	0.72 (0.04 – 11.93)	0.820
Complete primary education	-0.14 (-1.48 – 1.21)	0.87 (0.06 – 12.13)	0.918
Incomplete high school	2.1 (0.7 – 3.5)	8.16 (0.52 – 126.86)	0.134
Complete high school	0.56 (-0.65 – 1.77)	1.75 (0.16 – 18.71)	0.642
Incomplete Higher Education	-0.12 (-1.43 – 1.18)	0.88 (0.07 – 11.44)	0.925
Complete Higher Education	REF	-	-
Work			
Yes	REF	-	-
No	-0.71 (-1.21 - -0.22)	0.49 (0.18 – 1.3)	0.152
Fever days			
0	-	-	-
2	-1.61 (-2.6 - -0.62)	0.2 (0.03 – 1.38)	0.103
3	-1.34 (-2.09 - -0.6)	0.26 (0.06 – 1.12)	0.072
4	-1.11 (-1.86 - -0.36)	0.33 (0.08 – 1.44)	0.141
5	REF	-	-
Walking pain			
Yes	REF	-	-
No	-0.9 (-1.08 – 0.11)	0.62 (0.19 – 1.96)	0.412
Skin rashes			
Yes	REF	-	-
No	-0.84 (-1.4 - -0.28)	0.43 (0.14 – 1.3)	0.135
Anti-N test			
Fever days			
0	-	-	-
2	-	-	-
3	-1.2 (-1.8 - -0.6)	0.3 (0.09 – 0.98)	0.047
4	-0.92 (-1.52 - -0.32)	0.4 (0.12 – 1.28)	0.123
5	REF	-	-
Pruritus			
Yes	REF	-	-
No	-1.2 (-1.73 - -0.68)	0.3 (0.11 – 0.85)	0.023

4. Discussion

From 2019 to 2020, Maranhão State recorded 706 probable cases of chikungunya, although in 2020, there was a 74.8% reduction in cases compared with the cases reported in the previous year. This reduction can be attributed to the COVID-19 pandemic, which prevented epidemiological surveillance teams to work, causing delays or underreporting of arboviruses in Brazilian states. Moreover, a large proportion of the population feared seeking care in a health unit during this period.

In the context of the current pandemic, patients with COVID-19 can present similar clinical symptoms to arbovirus diseases, complicating the diagnosis and management of these patients. A previous study reported that some patients with COVID-19 may go unnoticed in settings with dual outbreaks, like COVID-19 and dengue [17].

A study in Peru revealed that the most frequent symptoms were headache (82.9%), myalgia (67.03%), and malaise (62.50%). Polyarthralgia in the hands and feet was significantly more common in patients with CHIKV infection, and conjunctivitis in patients with positive serology for SARS-CoV-2, similar to our results [18].

Our results suggest that CHIKV continues to circulate in the main regions of Maranhão. However, positive tests, using molecular biology for detection of CHIKV, have been unsatisfactory, with only three cases being identified out of 179 samples. This could have been due to insufficient and in time medical assistance to patients during the pandemic. Also, this lack of assistance coincided with the sample collection period, aside from the initial symptoms caused by the coronavirus being similar to those of the arboviruses DENV, CHIKV, and ZIKV, making clinical and laboratory diagnosis difficult. Because time of infection is of paramount importance for laboratory tests using molecular biology, many of those belatedly sought health assistance, losing the time of viremia when it was possible to identify viruses in the samples.

As the COVID-19 pandemic coincided with the resurgence of DENV in Brazil [19], the number of arbovirus cases fluctuated as simultaneous outbreaks occurred, generating a failure in epidemiological surveillance and leaving the health system unstable, making it almost impossible to detect DENV and CHIKV simultaneously.

However, when the presence of CHIKV, DENV, and ZIKV antibodies was investigated in the samples, our results revealed that the population had contact with some of these arboviruses. While COVID-19 cases were increasing, the arbovirus peak was decreasing, masking the outbreak, despite the DENV epidemic. The insufficient investigation, and incorrect diagnosis between dengue and COVID-19, which share similar symptoms in the early stages, masked the correct data on DENV in the year 2020 [20,21].

Our sociodemographic data also emphasize these findings, as many of the patients who tested serologically positive for CHIKV lived in unhealthy places with poor government assistance. As demonstrated by Mousavi [22], the poorest countries and the lowest socioeconomic groups were disproportionately more affected, as unfavorable conditions and the potential for DENV epidemics overlap with other infectious disease outbreaks.

5. Conclusions

Our results illustrate the situation of Maranhão State in the combat of the main arboviruses that affect our population. But unexpected challenges, such as the pandemic, led to failures in monitoring endemic diseases, and caused great damage to the health of the population. The social group with the lowest economic power, based on their socio-environmental condition, is the most vulnerable and mainly uses the public health system, therefore being the most prevalent in our research.

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