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Dengue Fever and *Aedes aegypti* in indigenous Brazilians: seroprevalence, risk factors, knowledge and practices

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Abstract

OBJECTIVE Dengue remains an important public health problem in Brazil. We estimated the associated factors of dengue seroprevalence among native Indians of the Tremembé ethnic and their knowledge about the aspects related to the presence of mosquitoes of the genus *Aedes*.

METHODS An analytical cross-sectional study and a prospective environmental study to monitor the trapping of mosquito eggs monthly were performed. The serological portion of the study involved indigenous people living in the village of Tapera in northeastern Brazil. Ovitraps were monitored for 12 months.

RESULTS Two hundred and ninety of 350 indigenous people (82.9%) participated in the study, with an average age of 30.2 years. The seroprevalence was 22.1% and positivity increased with age, with rates of 4.2% in children under 15 years of age, 26.8% in 15 to 59-year-olds and 42.3% in those older than 59 (CI: 2.25–15.96; $P < 0.001$). A higher incidence of moving to the city and the presence of underlying diseases were associated with the occurrence of dengue ($P < 0.001$). Four serotypes were detected, with the highest prevalence of DENV-1 (77.8%), followed by DENV-2 (70.4%), DENV-3 (14.8%) and DENV-4 (11.1%). Eggs were collected in all months of the year and in the traps located in the vicinities of the domiciles (57%).

CONCLUSIONS We present the first seroepidemiological survey of dengue conducted among indigenous populations in Brazil. This lack of studies is likely due to the great bureaucratic challenge of working with indigenous populations, which may lead to greater negligence in the health of these populations.

keywords dengue, epidemiology, indigenous, survey, *Aedes aegypti*

Introduction

Dengue remains an important public health problem in Brazil [1, 2]. In northeastern Brazil, the state of Ceará is characterised by the recurrent occurrence of epidemics since 1986 with an incidence of over 1000 cases/100 000 inhabitants and high mortality [3, 4], isolation of the four dengue serotypes [5] and cocirculation of the Chikungunya and Zika viruses since 2015 [6]. Information related to seroprevalence of dengue in Brazil is very old; even in large cities such as Fortaleza, the last published data refer to more than two decades ago [7, 8].

Indigenous people are among the vulnerable populations in Brazil and are usually excluded from dengue studies [9]. The Brazilian indigenous populations are among the largest in the world, with 230 ethnic groups and 180 languages [10–12]. Until 2014, there were no scientific publications or relevant information on dengue cases in these populations, until the first confirmed death of an indigenous Tremembé child occurred [13]. The denial of this case by the Indians themselves, due to information that there was no record of *Aedes aegypti* mosquitoes nor cases of dengue in that indigenous region [13], contributed to the demand for research in the

village area for a better understanding of viral circulation to reorient health services.

Thus, the objective of this work was to estimate the associated factors of dengue seroprevalence among native Indians of the Tremembé ethnic group of Tapera village and their knowledge about the aspects related to the presence of mosquitoes of the genus *Aedes*.

Methods

Ethical and legal aspects

The project was unanimously approved by the Local Council for Indigenous Health (CONLOSI), respecting Normative Ruling No. 001/PRES/1995/FUNAI for admission to indigenous lands for research purposes. Subsequently, as recommended by CNS Resolution 466/2012, the project was submitted and approved by CEP/CONEP, with opinion No. 956.213 and CAAE no 33411214.1.0000.5054.

Setting

The indigenous population of the Tremembé ethnic group, resident in the village of Tapera, is composed of approximately 350 individuals in 87 houses. The village belongs to the territory of the municipality of Itarema, in the northwest region of the state of Ceará (Figure 1). The relief is made up of coastal plains, established on Cenozoic sedimentary deposits. Although the climate is hot semi-arid tropical, there are periods of rainfall that are more frequent between the months of January and May. The village is bordered on the north by the mangrove/marsh of the Aracatí-mirim River, to the south and west by a large privately owned coconut plantation, and to the east by another village.

Study design

The study was developed in two stages. Initially, a cross-sectional analytical seroepidemiological survey was conducted using a semi-structured questionnaire, followed by a prospective environmental study of monthly monitoring of mosquito egg traps.

Serological survey

A 5-ml blood sample was collected to perform the tests in October and November 2015. Blood was collected by means of venoclysis, preferably by puncturing the median cubital, cephalic or basilic veins after identification by two-finger palpation, direct visualisation and asepsis of the chosen site.

After collection in a sterile tube with a clot activator and separator gel, the samples were individually labelled and stored in identified thermal boxes, cooled with frozen gel plates and sent directly to the Central Laboratory of Public Health of Ceará (LACEN-CE) for adequate storage and subsequent analysis. The samples were analysed for the presence of IgG antibodies against dengue virus (DENV) serotypes using the Panbio Dengue IgG Indirect ELISA®, according to the manufacturer's instructions. This test showed a serological sensitivity of 97.9% (CI 92.5–99.7%) for secondary dengue and 62.0% (CI 51.8–71.5%) for endemic dengue. The serological specificity was 100.0% (CI 96.6–100.0%) for dengue negatives. Confidence Interval used was 95%.

Concomitant to blood collection, a semi-structured questionnaire was applied to assess the level of knowledge of native Indians about the disease, its transmission, surveillance and control and to identify possible factors associated with the transmission of the disease.

Microneutralisation test

Dengue-1 virus (DENV-1 strain), Dengue-2 virus (DENV-2 New Guinea strain), Dengue-3 virus (DENV-3 H87 strain) and Dengue 4 virus (DENV-4 H241 strain) were kindly donated Dr. Benedito Antonio Lopes da Fonseca (Molecular Virology Laboratory, Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil).

All positive cases that presented a *cut-off* value >2000 by the Elisa IgG test were tested. The PRNT technique was performed only in a part of the sample because of the difficulty and cost of this technique. This test was performed at the Laboratory of Research on Pathogenic Bioagents of the University of Fortaleza/Experimental Biology Center (Unifor/Nubex), according to Vorndam & Beltran [14], with minor modifications. Vero cells (2×10^5) were distributed in 96-well plates, and primary antibodies (Miaf) from each DENV serotype and peroxidase-conjugated anti-IgG secondary antibodies (Sigma) were used. The optical density was read at a wavelength of 405 nm.

Entomological monitoring

A total of 42 houses were randomly selected for the installation of traps (ovitraps). Eighty-four traps were set, half inside houses and half outside in their vicinity (mean distance 3 m). Places inside the house with good ventilation and permanent circulation of people and animals were chosen, to increase the probability of a positive trap. Traps outside were preferably installed in places with greater circulation of people and sheltered from the rain.

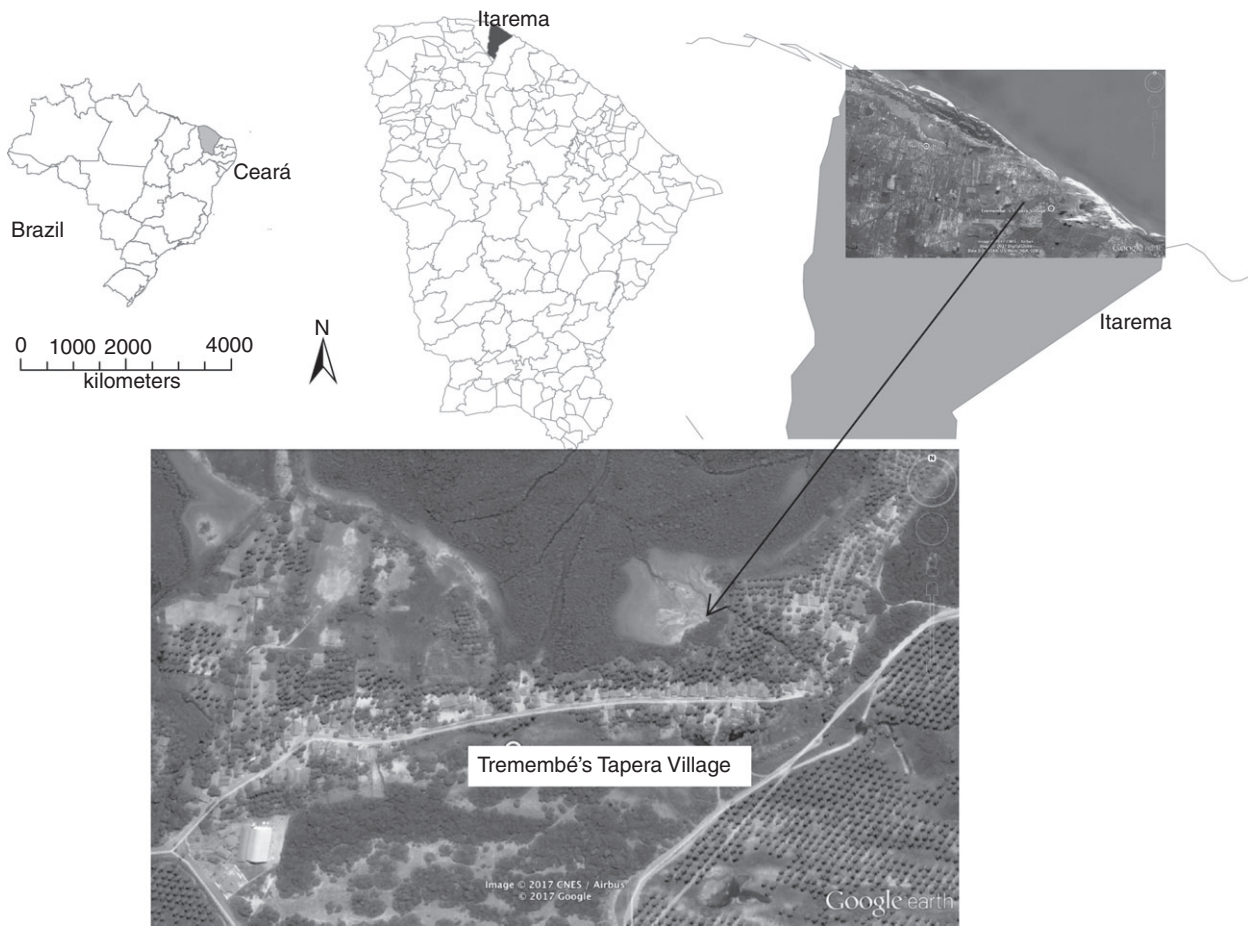

Figure 1 Village of Tapera in the municipality of Itarema, Ceará state, Brazil.

The traps were placed in the first two weeks of each month, and monitored for 12 months, beginning February of 2015 until January of 2016. After five to seven days, the traps were removed, and the vanes of the ovitraps were allowed to dry. They were then sent to the Laboratory of Medical Entomology of the School of Medicine of the Federal University of Ceará (UFC) to count the eggs and to evaluate their hatching in a controlled environment (temperature, humidity and photoperiod).

We recorded the number of residences that had at least one positive trap (RPT) and the number of positive traps (NPT). We also identified the highest number of eggs found in a residence (HNE) counting the number of eggs in each ovitrap. Two indexes were used to evaluate the mosquito infestation: the ovitrap positivity index (OPI) and the egg density index (EDI), the first one is calculated dividing the number of positive ovitraps by the total number of examined ovitraps and the second one by the number of eggs divided by number of positive ovitraps [15, 16].

Data analysis

Data were entered and analysed using Epiinfo® software version 3.5.1 (Centers for Disease Control and Prevention, USA) [17]. The association between the outcome (serological screening results – IgG) and the epidemiological variables investigated was analysed using contingency tables with prevalence ratios and were tested by the Pearson's chi-squared test or Fisher's exact test, with a confidence interval of 95% and with statistical significance defined as $P < 0.05$. The entomological data were presented descriptively.

We selected those variables that presented statistical significance with a P value less or equal to 0.25 for the multivariable analysis. We used a generalised linear model (GLM) with logarithmic link and Poisson

distribution family to estimate the relative risk and confidence intervals by using robust error variances to evaluate the differences between categories of explanatory variables for the outcome in final model [18].

Results

Participants

A total of 290/350 indigenous people (82.9%) participated in the study, with an average age of 30.2 years and deviation standard of 19.5. The majority (65.1%) were in the economically active age (15 to 59 years), 62.0% were illiterate or with little schooling and 62.0% of families were surviving with monthly incomes below the minimum wage. Relocations to the city were infrequent, with frequent visits to the social benefits office (Table 1).

Most houses had family nuclei composed of at least six dwellers, were made of masonry (95.7%) and had a regular water supply (77%). The main destination of household waste was burning (44.4%).

Prevalence and associated factors

The seroprevalence was 22.1% (62/280), of which 39 cases were women (63%) and 23 were men (37%). with rates of 4.2% in children under 15 years of age, 26.8% in 15 to 59-year-olds and 42.3% in those older than 59 (CI: 2.25–15.96; $P < 0.001$). Only four individuals (4.9%) younger than 15 years old were positive (Table 2).

Seroprevalence of DENV was significantly associated with the following variables: the thought that one has dengue (CI: 1.38–5.41), the presence of underlying diseases (hypertension and diabetes) (CI: 1.41–3.34), routine medication intake (CI: 1.67–4.18), a greater frequency of trips to the city (CI: 0.21–0.78) and the presence of mosquitoes at home (CI: 1.02–2.47) (Table 3). Recognition of control measures was not associated with having dengue. Additionally, previous infection was not associated with family income, house type, houses with insect protection, water supply sources, habit of storing water or waste destination (Table 2).

Table 1 Sociodemographic aspects of Tremembé Indigenous, Tapera Village, Itarema, Ceará, Brazil

Sociodemographic aspects	No	%
Age group (281)		
<15 years	72	25.6
15–59 years	183	65.1
>59 years	26	9.0
Education (276)		
Low education / illiterate	171	62.0
Reasonable education	52	18.8
Good education	53	19.2
Occupation (278)		
Homework or Unemployed	110	39.6
Student	75	27.0
Working outside the home	69	24.8
Retired	24	8.6
Family income (224)		
Up to 1 minimum wage	140	62.5
>1 minimum wage	84	37.5
Frequency of trips to city (241)		
Up to 3 times a week	85	35.3
Less than 3 times a week	141	58.5
Almost never	15	6.2
Vaccinated against yellow fever (279)		
Yes	256	91.8
No	23	8.2

Table 2 Sociodemographic aspects of Tremembé Indigenous after seroepidemiological survey of dengue in Tapera village, Itarema, Ceará

Sociodemographic variables	Positive no (%)	Negative no (%)	RP	IC
Sex				
Female	39 (23.8)	125 (76.2)	0.83	0.53–1.32
Male	23 (19.8)	93 (80.2)		
Age group*†				
<15 years	3 (4.2)	69 (95.8)	5.99	2.25–15.96
15–59 years	48 (26.8)	131 (73.2)		
>59 years	11 (42.3)	15 (57.7)		
Family income				
Up to 1 minimum wage	32 (22.9)	108 (77.1)	1.23	0.77–1.95
>1 minimum wage	23 (28.1)	59 (71.9)		
Residing in masonry house†				
Yes	56 (21.3)	207 (78.3)	0.69	0.29–1.62
No	4 (30.8)	9 (69.2)		
Insect protection at home				
Yes	11 (14.5)	65 (85.5)	0.59	0.32–1.07
No	49 (24.6)	15 (75.4)		
Water supply				
Public (SESANI)	44 (21.2)	164 (78.8)	1.12	0.67–1.88
Others	15 (23.8)	48 (76.2)		
Habit to store water				
Yes	45 (22.2)	158 (77.8)	1.03	0.62–1.74
No	15 (21.4)	55 (78.6)		
Waste destination				
Systematic collection	22 (23.4)	72 (76.6)	0.85	0.53–1.36
Others	36 (20.0)	144 (80.0)		

*Statistical significance.
†Fisher exact.

Table 3 Behavioural and risk aspects of Tremembé indigenous after seroepidemiological survey of dengue in the village Tapera, Itarema, Ceará

Behavioural variables	Positive no (%)	Negative no (%)	RP	IC
You think you had dengue*†				
Yes	4 (57.1)	3 (42.9)	1	–
No	56 (20.9)	212 (79.1)	2.73	1.38–5.41
Know dengue symptoms				
Yes	43 (24.6)	132 (75.4)	1.13	0.67–1.89
No	15 (21.7)	54 (78.3)		
Vaccinated against YF				
Yes	53 (19.3)	200 (79.1)	0.58	0.32–1.05
No	8 (36.4)	14 (63.6)		
Chronic disease (hypertension, diabetes, etc.)*				
Yes	24 (38.1)	39 (61.9)	2.17	1.41–3.34
No	37 (17.5)	174 (82.5)		
Continued use of medications*				
Yes	33 (37.5)	55 (62.5)	2.64	1.67–4.18
No	24 (14.2)	145 (85.8)		
Displacement for city*				
Often	18 (21.2)	67 (78.8)	0.40	0.21–0.78
Very little	13 (8.5)	140 (91.5)		
It reports the presence of mosquito				
Yes	30 (28.9)	74 (71.1)	1.59	1.02–2.47
No	30 (18.2)	135 (81.8)		
There are preventive measures				
Yes	52 (21.4)	181 (78.6)	0.90	0.40–2.00
No	5 (23.1)	16 (76.2)		

*Statistical significance.
†Fisher exact.

Table 4 Positivity of microneutralisation tests for Tremembé dengue in Tapera village, Itarema, Ceará

Sex	Age	DENV-1	DENV-2	DENV-3	DENV-4
F	12	P	N	N	N
F	16	P	P	N	N
F	19	P	P	N	N
F	20	P	P	N	N
F	21	P	P	N	P
M	24	P	N	N	N
F	26	P	N	N	N
M	26	N	P	P	N
F	27	P	P	N	N
F	27	N	P	P	N
F	30	P	N	N	N
F	33	P	P	N	N
F	35	N	N	P	N
F	36	P	P	N	N
M	41	P	P	N	N
F	44	N	P	N	N
F	45	P	P	N	N
M	45	P	P	N	P
M	47	P	P	N	N
M	48	N	P	N	N
F	52	P	N	N	N
F	53	P	P	N	N
M	61	P	P	N	N
M	62	N	P	P	P
F	64	P	P	N	N
F	78	P	N	N	N
M	83	P	N	N	N

M, male; F, female; P, positive; N, negative.

Three variables remained in the GLM model. Being 15 years of age or older had a RR = 7.79 (CI 95%: 1.02–59.34; $P = 0.047$). The second variable associated with the prevalence of dengue fever was leaving the village with RR = 2.29 (CI 95%: 1.09–4.82; $P = 0.029$), and the last one was continuous use of medication with a RR = 2.13 (CI 95%: 1.01–4.48).

Microneutralisation

Among the 62 positive cases, 27 (43.5%) were tested by the microneutralisation test. DENV-1 showed the highest presence (77.8%), followed by DENV-2 (70.4%), DENV-3 (14.8%) and DENV-4 (11.1%). None of the 27 indigenous people tested had dengue four times, while 10 (37.0%) had dengue once, 14 (51.9%) were infected twice and three (11.1%) had dengue three times (Table 4). The median age of these patients was 33 years (ranging from 12 to 83), with 96.3% being older than 15 years.

Knowledge by Tapera's indigenous

Television programmes were the main source of information acquisition on dengue, being highlighted by 57.7% of the population, while lectures (40.5%) and visits by indigenous health workers were cited by less than half the indigenous population (35.9%).

Fever was the most recognised symptom of dengue by indigenous people (82.7%), followed by pain in the muscles (66.2%), eyes (63.4%), and joints (57.6%) and vomiting (57.0%). The recognition of symptoms by the indigenous people was not statistically associated with seroprevalence.

The most cited control measures were to eliminate standing water (76.4%), to place sand in plant pots (76.4%), to eliminate litter (60.2%) and to clean their backyards (50.4%). Alternatives such as using insecticides or the use of larval fish in water reservoirs were recalled by only 24.3% and 15.8% of the indigenous people, respectively.

Entomological aspects

Eggs were collected in all months of the year, with the highest number in March (3145; 17.5% of eggs) and the lowest number in September (379 eggs). The highest concentration of eggs collected occurred between February and August (81%). The number of eggs collected in traps located in the vicinities of the domiciles was higher (57%) than that collected within the domiciles. The differences in the numbers of eggs per site of installed trap were more dissimilar in the months of September and October. Eggs were collected in all houses in at least one of the traps. In six of the houses, there were traps with eggs during 91.7% of the monthly inspections, and half of the houses had detectable mosquito eggs in the traps for nine months (Table 5).

The highest number of residences positive trap (RPT) was in March 2015 (39). This month also had the highest number of positive traps (NPT) and the highest number of eggs (HNE - 459) (Table 5).

Discussion

We present the first seroepidemiological survey of dengue conducted among indigenous populations in Brazil. It is possible that other minor works have been performed, but not covering a complete village. This lack of studies is likely due to the great bureaucratic challenge of working with indigenous populations, which may lead to greater negligence of the health of these populations.

It is estimated that when Europeans arrived in Brazil, there were more than five million indigenous people; in 2016, this figure has shrunk to just over 800 000 [10]. Thus, all efforts to identify possible diseases and risk factors should be encouraged to reduce deaths in these historically disadvantaged populations, especially with regard to infant mortality or preventable causes.

The Tremembé people were persecuted for more than two centuries. From the 1980s, they began to reappear and to undergo a process of re-ethnicisation and rediscovery of their origins [19]. Despite losing much of the phenotype that would identify them with Amazonian Indians, they still maintain rites, customs and habits common to indigenous peoples in the Northeast.

The social and economic situation of the Tremembé of Tapera village portrays the reality of the indigenous peoples of the Brazilian Northeast. Different from the indigenous population in the North, where the predominant age group is below 15 years of age, in the Northeast, the economically active range predominates (65%) [10].

It is noteworthy that a large part of the income comes from social programmes such as Bolsa Família; in 2014, two/three of the Brazilian indigenous population was living based on this support [20]. In spite of their poverty, it is possible that this situation will change in the next years, as all of the 165 Tremembé children of school age in Tapera village attended the school at the time of this research.

Among the natives who participated in the study, there was a predominance of females in both questionnaire responses and blood collections, a fact similar to that reported in other studies with non-indigenous populations. In fact, women, even indigenous women, appear to be more assiduously attending health services and to better adhere to the proposed treatments and activities [21, 22]. Despite the greater number of women, there was no significant difference in seroprevalence in relation to sex.

Table 5 Number of eggs collected, by trap location, in the village Tapera, Itarema, Ceará

	Inside house		Outside house							
Months/location	No	%	No	%	RPT	NPT	HNE	OPI	EDI	Total off eggs no (%)
Feb 2015	394	38.9	619	61.1	34	52	104	19.5	61.9	1013 (5.6)
Mar 2015	1537	48.9	1608	51.1	39	68	459	46.3	81.0	3145 (17.5)
Apr 2015	638	31.7	1377	68.3	39	66	168	30.5	78.6	2015 (11.2)
May 2015	1094	55.7	869	44.3	34	58	161	33.8	69.0	1963 (10.9)
Jun 2015	753	35.9	1343	64.1	37	60	261	34.9	71.4	2096 (11.6)
Jul 2015	797	41.9	1103	58.1	37	60	349	31.7	71.4	1900 (10.6)
Aug 2015	1082	45.6	1292	54.4	36	62	253	38.3	73.8	2374 (13.2)
Sep 2015	247	65.2	132	34.8	21	24	60	15.8	28.6	379 (2.1)
Oct 2015	447	25.3	1321	74.7	26	40	420	44.2	47.6	1768 (9.8)
Nov 2015	391	55.4	315	44.6	27	37	138	19.1	44.0	706 (3.9)
Dec 2015	361	57.0	272	43.0	24	33	164	19.2	39.3	633 (3.5)
Jan 2016	182	40.3	270	59.7	21	26	59	17.4	31.0	452 (2.5)
Total	7741	43.0	10 251	57.0	–	–	–	–	–	17 992 (100.0)

RPT, residences positive trap; NPT, number of positive traps; HNE, highest number of eggs; OPI, ovitraps positivity index; EDI, egg density index.

An increase in age showed a significant increase in exposure to the virus; however, there is not enough evidence to explain this finding besides the longer exposure time.

Among those who frequently visit the municipality of Itarema, little more than one-fifth presented seropositivity for DENV, *vs*. fewer than 10% of those who rarely go to the city. Formal workers, retirees and beneficiaries of social programmes (or their legal representatives) had reason to visit the town hall of Itarema regularly. These trips may place them at increased risk of dengue infection [23–26].

Among the indigenous people who cited some form of avoiding the proliferation of the main transmitting mosquito, the most remembered methods were to eliminate standing water and to put sand in plant pots, which were reported by almost 90% of the people. The fight against dengue is a recurring issue, and many Tremembé remember the forms of prevention and main symptoms but still neglect the simplest forms of combat. Buckets and tanks are the main form of water storage the indigenous people despite having a guaranteed water supply, either directly or through wells.

Storing water in pots, tanks and small deposits seems to be a cultural and ancient habit. The main reasons cited were fear of lack of water and lack of adequate structures in many houses to support the weight of a water tank. The evidence that the cultural aspect is the strongest is clearly perceived, as neither justification is based on the current reality of most houses; 95.7% of the Tremembé of this village live in masonry houses, and almost all admit that lack of water is a rare event, not remembering the last time they were without water for more than a day. Culturally, this behaviour is justified by the habit of taking cold baths, which, according to them, is impossible with tank water because the water is warmed by solar irradiation. Thus, even those who have running water, water tanks and enough information make the choice to keep water in tanks, drums and buckets, facilitating mosquito oviposition [27].

Properly disposing of garbage was the third most cited measure by indigenous people as a protective measure. However, this strategy seems to be fundamental to the success of any attempt at environmental control [28]. Many still choose to burn their garbage, and few people bury it. These strategies are not efficient for eliminating deposits capable of storing rainwater.

The Tremembé people consider the information transmitted by health professionals to be quite reliable, but more than half of them recognise television as the main disseminator of information in the village. Almost all homes have at least one television, which stays on as long as someone is at home. However, many admitted to not paying much attention to government-produced commercials. This fact is not in line with other studies conducted with non-indigenous populations [22, 29, 30].

The prevalence detected should be considered high since there are no reports of dengue in indigenous populations. Indeed, if compared to surveys conducted in larger and more densely populated cities, the prevalence does not seem to be very high. However, if compared with results found in surveys conducted in rural areas with lower population densities, the seroprevalence in Tapera could be considered high, as the prevalence ranged from 12 to 17% in similar areas. However, it is important to consider the relative timeline since all of the studies compared here are more than 15 years old [31–34]. Knowing how to recognise the initial symptoms of dengue is an important factor in deciding whether or not to seek early medical help, which can have a great impact when the final outcome is considered – 'remission and cure' or 'death' – and in the adoption of early sanitation measures [22, 29, 35, 36]. The aspect already observed in other studies with non-indigenous populations is that the majority of natives with positive results for prior infection by DENV did not perceive the symptoms of a previous exposure to DENV or had asymptomatic disease. Among the 60 positive cases, only four had said they thought they had had dengue. This result confirms the findings of asymptomatic or oligosymptomatic clinical infection, very common in cases of first DENV infection, and highlights the population's low capacity to positively identify general and non-specific symptoms for viral infections in a tropical context [37–41]. Another aspect worth mentioning was the fact that the microneutralisation results confirmed the higher prevalence of positive tests in older people.

Aedes aegypti is known for its strongly anthropophilic behaviour. The habit of storing water and the natural or artificial formation of cavities that retain water allowed a vast exhibition of breeding places throughout the village. More than 17 000 eggs were collected, a value justified in part by the large number of traps installed.

Mosquitoes of the genus *Aedes* were found throughout the village, taking into account the average flight autonomy, which, in rural areas, is approximately 570 metres [42]. Thus, the whole village could be considered infested, although individually, some houses presented better conditions of physical or environmental control. This infestation may have been influenced by climatic factors such as the annual mean temperature of 27.5 °C (26.8–28.3 °C) and rainfall concentrated in the months of January to May. The rainy season starts in December and can goes up to July. In the months of higher rainfall, it is possible to justify the reduction of egg laying in the traps because other attractive sites for oviposition exist, which compete directly with the traps. Because of these

environmental conditions, the Tremembé people of Tapera village keep the doors and windows of their houses constantly open to increase ventilation. This habit should also contribute to a more efficient dispersion of the vectors, even with houses spaced apart from each other and the very strong winds due to the village's geographic location near the beaches and dunes.

The ovitrap positivity index increased during the months of March to August, indicating a greater spatial distribution of eggs. This information is important because it indicates the period when there is a greater likelihood of contact between people and insects, favouring transmission.

It is difficult to pinpoint when the subjects became ill with dengue fever, but it could have been many years earlier. But now the presence of mosquito's eggs in their houses and surrounding areas cannot be associated with their convalescence, as it is impossible to know where the subjects lived when they became infected or what the then mosquitoes ecology was in those places.

We recognise the need for bureaucracy to be involved in research with indigenous populations in Brazil, but we believe that this understanding should be encouraged to provide better health services suited to the people's needs and their specificities. Recognising that dengue fever circulates among indigenous people is essential to guide health professionals working with these populations and to improve the differential diagnosis of some of the most prevalent diseases in the village. These results suggest that dengue can be present in other villages and ethnicities, requiring specific interventions and public policies.

Limitations of the study

The PRNT technique was performed only in a part of the sample (43.5%) because of the difficulty and cost of this technique. During the initial months of egg collection, some vanes were stored inadequately, causing humidity and consequent hatching of some eggs. Another aspect that limited the execution of the work was the long time between the first contact with the indigenous people, after confirmation of the first death of the Tremembé child, and the beginning of the experiments due to the ethical release procedures of the official bodies.

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