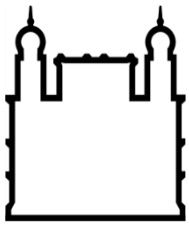

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INSTITUTO GONÇALO MONIZ

FIOCRUZ

Curso de Pós-Graduação em Biotecnologia em Saúde e Medicina Investigativa

TESE DE DOUTORADO

CARACTERÍSTICAS CLÍNICAS E EPIDEMIOLÓGICAS ASSOCIADAS À INFECÇÃO POR DENGUE, ZIKA E CHIKUNGUNYA EM SALVADOR, BAHIA.

MONAISE MADALENA OLIVEIRA E SILVA

Salvador - Bahia
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Orientador: Professor Dr. Guilherme de Sousa Ribeiro

Tese apresentada ao Curso de Pós-Graduação em Biotecnologia em Saúde e Medicina Investigativa da Fiocruz-IGM para obtenção do título de Doutor.

Salvador - Bahia
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" CARACTERÍSTICAS CLÍNICAS E EPIDEMIOLÓGICAS ASSOCIADAS A INFECÇÃO POR DENGUE, ZIKA E CHIKUNGUNYA EM SALVADOR, BAHIA."

MONAISE MADALENA OLIVEIRA E SILVA

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Dedico a minha cidade natal: Ipirá-Ba. Que também foi acometida por epidemia de Chikungunya e Zika, causando muito sofrimento e sequelas duradouras à população.

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“Combati o bom combate, acabei a carreira, guardei a fé.”

2º Timóteo. 4-7

SILVA, Monaise Madalena Oliveira. Características clínicas e epidemiológicas associadas à infecção por Dengue, Zika e Chikungunya em Salvador, Bahia. 79 f. il. Tese (Doutorado em Biotecnologia em Saúde e Medicina Investigativa) – Fundação Oswaldo Cruz, Instituto Gonçalo Moniz, Salvador, 2018.

RESUMO

INTRODUÇÃO: Dengue é endêmico no Brasil desde 1981. O primeiro caso com transmissão autóctone de infecção por vírus Chikungunya (CHIKV) no Brasil foi confirmado em 2014, chamando a atenção das autoridades sanitárias, principalmente, devido a cronificação da artralgia. A partir de 2015 a circulação do vírus Zika (ZIKV) foi confirmada no país. **OBJETIVO:** Investigar aspectos epidemiológicos e clínicos de pacientes com infecções por ZIKV, DENV e CHIKV, e o processo de cronificação dos sintomas articulares dos pacientes confirmados por Chikungunya. **MÉTODO:** De setembro de 2014 a julho de 2016 foi realizado um estudo de vigilância para doença febril, em um centro de emergência em saúde de Salvador, para identificação de pacientes com idade ≥6 meses, que referiram febre nos últimos 7 dias ou que apresentaram temperatura ≥37,8ºC durante o atendimento. Entrevistamos os pacientes para coletar dados demográficos e clínicos, e revisamos os prontuários para obter a suspeita diagnóstica. Amostras de sangue de fase aguda e convalescente foram coletadas. Realizou-se testes moleculares e sorológicos para confirmar o diagnóstico de DENV, CHIKV, ZIKV ou Flavivírus não específico. Os participantes com confirmação laboratorial para CHIKV foram acompanhados via contato telefônico, em média, um ano e cinco meses após a fase aguda da doença, a fim de investigar a evolução do quadro clínico e fatores de risco associados a cronificação da artralgia. **RESULTADOS:** Dos 948 participantes inclusos, 247 (26,1%) tinham evidência laboratorial de uma infecção arboviral, dos quais 224 (23,6%) eram infecções simples (DENV: 32, 3,4%; CHIKV: 159, 16,7%; ZIKV: 13, 1,4% e Flavivírus: 20, 2,1%), e 23 (2,4%) foram co-infecções (DENV / CHIKV: 13, 1,4%; FLAV / CHIKV: 9, 0,9%; e DEN / ZIKV: 1, 0,1%). Rash e prurido foram mais frequente em pacientes com infecção por ZIKV, e artralgia foi mais comum em pacientes com infecção por CHIKV. Dos 265 pacientes confirmados com infecção por CHIKV, 153 (57,7%) foram acompanhados por contato telefônico, e destes, 65 (42,5%) referiram artralgia crônica, e 47 (30,7%) estavam sintomáticos no momento do contato telefônico. Sexo feminino e idade estão associados ao risco de cronificação da artralgia. **CONCLUSÃO:** Nossos achados revelam um desafio para um diagnóstico clínico preciso de infecções por DENV, CHIKV e ZIKV em uma área de co-circulação, além disso, co-infecções são eventos frequentes. Destacamos a alta frequência da dor articular persistente após uma infecção por CHIKV, e o impacto da artralgia crônica nas atividades diárias e laborais dos pacientes.

Palavras-Chaves: Epidemiologia, Arboviroses, Cronificação.

SILVA, Monaise Madalena Oliveira. Clinical and epidemiological characteristics associated with Dengue, Zika and Chikungunya infection in Salvador, Bahia. 79 f. il. Thesis (Ph.D em Biotecnologia em Saúde e Medicina Investigativa) - Oswaldo Cruz Foundation, Institut Gonçalo Moniz, Salvador, 2018.

ABSTRACT

INTRODUCTION: Dengue has been endemic in Brazil since 1981. The first case with autochthonous transmission of Chikungunya (CHIKV) virus infection in Brazil was confirmed in 2014, drawing attention of the health authorities, especially due to arthralgia chronification. Since 2015, the circulation of Zika virus (ZIKV) was confirmed in the country. **AIM:** To investigate the epidemiologic and clinical aspects of patients with ZIKV, DENV and CHIKV infection and the process of chronification of the joint symptoms of the patients confirmed for Chikungunya. **METHOD:** From September 2014 to July 2016, a surveillance study for febrile illness was carried out at a health emergency center in Salvador, to identify patients aged ≥6 months who reported fever in the last 7 days or who presented a temperature ≥ 37.8ºC during medical care. We interviewed the patients to collect demographic and clinical data. In addition, we reviewed the medical records to have the diagnostic suspicion. Acute and convalescent phase blood samples were collected. Molecular and serological tests were performed to confirm the diagnosis of DENV, CHIKV, ZIKV or non-specific Flavivirus. Participants with laboratory confirmation for CHIKV were assisted through telephone, on average, one year and five months after the acute stage of the disease to investigate the evolution of clinical picture and risk factors associated with arthralgia chronification. **RESULTS:** Of the 948 participants included, 247 (26.1%) had laboratory evidence of an arboviral infection, of which 224 (23.6%) were simple infections (DENV: 32, 3.4%, CHIKV: 159, 16.7%) and 23 (2.4%) were co-infections (DENV / CHIKV: 13, 1.4%, FLAV / CHIKV: 9, 0.9%, and DEN / ZIKV: 1, 0.1%). Rash and pruritus were more frequent in patients with ZIKV infection, and arthralgia was more common in patients with CHIKV infection. From the 265 patients confirmed with CHIKV infection, 153 (57.7%) were assisted by telephone and, among them, 65 (42.5%) reported chronic arthralgia and 47 (30.7%) remained symptomatic until the moment they were contacted. Female sex and age are associated with the risk of arthralgia chronification. **CONCLUSION:** Our findings reveal a challenge for an accurate clinical diagnosis of DENV, CHIKV and ZIKV infections in a co-circulation area, in addition, co-infections are frequent events. We highlight the high frequency of persistent joint pain after a CHIKV infection, and the impact of chronic arthralgia on patients' daily and work activities.

Key words: Epidemiology, Arboviruses, Chronification

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LISTA DE ABREVIATURAS

OMS	Organização mundial de saúde
WHO	World Health Organization
M.S	Ministério da Saúde
I.C	Intervalo de confiança
DENV	Dengue Vírus
CHIKV	Chikungunya Vírus
ZIKV	Zika Vírus
DFA	Doença febril aguda
CSSM	Centro de Saúde de São Marcos
TCLE	Termo de consentimento livre e esclarecido
TALE	Termo de assentimento livre e esclarecido
CEP	Comitê de ética e pesquisa
Fiocruz	Fundação Oswaldo Cruz
CPqGM	Centro de Pesquisa Gonçalo Muniz
IgM	Imunoglobulina M
PCR	Reação em Cadeia Polimerase
Elisa	Enzyme Linked Immunoabsorbent Assay
RR	Risco Relativo
IIQ	Intervalo interquartil
NS1	Proteína não estrutural 1

SUMÁRIO

1 INTRODUÇÃO

O vírus da Dengue (DENV), o vírus da Zika (ZIKV) e o vírus da Chikungunya (CHIKV) são arbovírus que tem como via principal de transmissão mosquitos hematófagos, como o *Aedes aegypti* e *Aedes albopictus* (TRAVASSOS *et al*, 1998; WHO, 1967).

No Brasil, a primeira epidemia de dengue documentada clinicamente e laboratorialmente, ocorreu em 1981-1982 (OSANAI, 1983). O DENV se mantém em transmissão no país com picos epidêmicos, chamando a atenção das autoridades sanitárias brasileiras, da população e dos profissionais de saúde pela magnitude da doença e a dificuldade do controle do vetor (WHO, 2009).

O dengue reemergiu no Estado da Bahia em 1994, sendo crescente a incidência de dengue, de modo semelhante como aconteceu no Brasil (MELO *et al*, 2010; TEIXEIRA *et al*, 2001). Em Salvador (BA), as primeiras epidemias de dengue ocorreram em 1995 e 1996, desde este período a transmissão do DENV vem sucedendo no município, de forma endêmica, intercalada com períodos de epidemia (TEIXEIRA *et al.,* 2001).

Os primeiros casos com transmissão autóctone de infecção por CHIKV foram detectados no Brasil em 2014, na Região Norte (Amapá) e Nordeste (Bahia) (TEIXEIRA *et al*, 2015; ZEANA *et al*., 2016). Segundo dados da Secretaria Municipal de Saúde de Salvador, casos de infecção por Chikungunya foram notificados a partir de 2014 na cidade de Salvador (BRASIL, 2015).

O CHIKV tornou-se uma ameaça de grande magnitude devido a capacidade da doença produzir dores articulares intensas e persistente nos pacientes (BRIGHTON *et al*, 1983; JEANDEL *et al*, 2004; WAYMOUTH *et al*, 2013; DIAS *et al*, 2018). Estudos descrevem que 60% dos pacientes com infecção confirmada por chikungunya relataram sintomas por mais de três meses, sendo a artralgia a sintomatologia mais frequente, causando prejuízo na qualidade de vida dos indivíduos (BOGHERINE *et al*, 2008; ARROYO-AVILA *et al*, 2018). Além disso, pesquisas demonstram que a presença de sinais de depressão e/ou humor deprimido é frequente após a infecção por CHIKV (SCHILTE *et al*, 2013; SOMAHORO *et al*, 2009).

Em 2014, ocorreu o primeiro registro de caso suspeito de ZIKV no Rio Grande do Norte, entretanto, apenas em 2015, o Brasil confirmou o primeiro caso autóctone de infecção por Zika vírus em uma cidade da Bahia (CAMPOS *et al*., 2015; ZANLUNCA *et al.*, 2015). Em Salvador, dados epidemiológicos indicam que o surto de ZIKV começou em fevereiro/2015 (CARDOSO *et al.*, 2015).

Nos humanos, infecções causadas por arbovírus em geral apresentam sintomas iniciais inespecíficos e similares como: febre, prostração, dor articular, dor de cabeça, dor muscular, dor retro-orbital, calafrio, tontura, náusea, exantema e dor abdominal. (BRASIL, 2016; VASCONCELOS *et al*, 1992; VASCONCELOS *et al*, 1998; SCHWARTZ, 2010; SISSOKO *et al*, 2008; PETTERSEN *et al*, 2016). Estudos destacam que a dor articular é uma sintomatologia mais frequente em pacientes com infecção por chikungunya, exantema é mais proeminente em infecções por Zika vírus, e prostração é mais comum em infecções por dengue, quando comparada as manifestações clínicas entre pacientes com infecção por DENV, ZIKV e CHIKV (AZEREDO *et al*, 2018; SAHADEO *et al*, 2015).

Os métodos diagnósticos laboratoriais para confirmação da infecção por arbovírus são de alto custo e não estão disponíveis nos serviços de saúde. Ademais, a técnica baseada em detecção de anticorpos tem limitações, pois pode ocorrer reação cruzada para outros vírus endêmicos pertencentes à mesma família, a exemplo da ZIKV e DENV no Brasil, ambos flavivírus (WAGGONER *et al*, 2016a).

Devido a co-circulação dessas arboviroses com um quadro clínico similar e as dificuldades laboratoriais existentes, entende-se a necessidade de melhor compreender as semelhanças e diferenças entre as manifestações clínicas, e a epidemiologia do DENV, CHIKV e ZIKV. Além disso, é importante investigar o risco de cronificação da artralgia dos pacientes com confirmação laboratorial de infecção por CHIKV, e o possível impacto na vida dos indivíduos decorrente do processo de cronificação da dor articular.

Esse estudo contribuirá para o avanço do conhecimento científico sobre as doenças arbovirais, e ajudará entender a epidemiologia da DENV, CHIKV e ZIKV, auxiliando a guiar ações de detecção e controle de epidemias. Nossos achados descreverão semelhanças e diferenças entre as manifestações clínicas dessas três arboviroses, e ajudarão no direcionamento do cuidado clínico.

O seguimento dos pacientes com infecção por chikungunya ajudará a estabelecer o risco de cronificação da artralgia, fatores de risco associados, e qual o impacto da dor articular persistente no cotidiano da vida das pessoas.

Adicionalmente nossos dados fornecerão resultados laboratoriais que ajudarão na confirmação dos casos suspeitos notificados pelo sistema de vigilância.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Estudar aspectos epidemiológicos e clínicos de pacientes com infecções por DENV, CHIKV e ZIKV.

2.2 OBJETIVOS ESPECÍFICOS

- Estimar a frequência de infecção laboratorialmente confirmada por DENV, CHIKV e ZIKV entre pacientes que procuraram atendimento médico por doença febril em uma Emergência de Saúde em Salvador;
- Monitorar a distribuição temporal de infecções laboratorialmente confirmada por DENV, CHIKV e ZIKV em Salvador-BA no período de 2014-2016;
- Identificar semelhanças e diferenças entre as características demográficas, e clínicas de pacientes com infecção laboratorialmente confirmada por ZIKV, CHIKV, DENV.
- Estimar o risco de artralgia crônica em pacientes com confirmação laboratorial de infecção por CHIKV, e avaliar os fatores de risco associados.

3 RESULTADOS

Os resultados desta tese serão apresentados nos dois manuscritos a seguir:

3.1 MANUSCRITO 1- Concomitant transmission of dengue, chikungunya and Zika viruses in Brazil: Clinical and epidemiological findings from surveillance for acute febrile illness.

O manuscrito 1 foi submetido à revista *Clinical Infectious Diseases* no formato de artigo, e corresponde aos objetivos específicos da tese:

- Estimar a frequência de infecção laboratorialmente confirmada por DENV, CHIKV e ZIKV entre pacientes que procuraram atendimento médico por doença febril em uma Emergência de Saúde em Salvador;
- Monitorar a distribuição temporal de infecções laboratorialmente confirmada por DENV, CHIKV e ZIKV em Salvador-BA no período de 2014-2016;
- Identificar semelhanças e diferenças entre as características demográficas, e clínicas de pacientes com infecção laboratorialmente confirmada por ZIKV, CHIKV, DENV.

Concomitant transmission of dengue, chikungunya and Zika viruses in Brazil: Clinical and epidemiological findings from surveillance for acute febrile illness

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Keywords. Dengue virus, chikungunya virus, Zika virus, arbovirus, co-infection.

Running title. DENV, CHIKV, and ZIKV infections, Brazil.

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Summary. Simultaneous transmission of dengue, chikungunya, and Zika viruses occurs in endemic regions, leading to frequent co-infections. Rash and pruritus are more common with Zika, while arthralgia and swollen joints are more common with chikungunya. Nevertheless, correct medical diagnosis is challenging.

ABSTRACT

Background. Since their emergence in the Americas, chikungunya (CHIKV) and Zika (ZIKV) viruses co-circulate with dengue virus (DENV), hampering clinical diagnosis. We investigated clinical and epidemiological characteristics of arboviral infections during the introduction and spread of CHIKV and ZIKV through Brazil.

Methods. Surveillance for arboviral diseases among febrile patients was performed at an emergency health unit of Salvador, Brazil between Sep/2014-Jul/2016. We interviewed patients to collect data on symptoms, reviewed medical records to obtain the presumptive diagnoses, and performed molecular and serological testing to confirm DENV, CHIKV, ZIKV, or non-specific flavivirus (FLAV) diagnosis.

Results. Of 948 participants, 247 (26.1%) had an acute infection, of which 224 (23.6%) were single infections (DENV: 32, or 3.4%; CHIKV: 159, 16.7%; ZIKV: 13, 1.4%; and FLAV: 20, 2.1%), and 23 (2.4%) co-infections (DENV/CHIKV: 13, 1.4%; CHIKV/FLAV: 9, 0.9%; and DEN/ZIKV: 1, 0.1%). Additional 133 (14.0%) patients had serological evidence for a recent arboviral infection. Patients with Zika presented rash (69.2%) and pruritus (69.2%) more frequently than those with dengue (37.5% and 31.2%, respectively) and chikungunya (22.9% and 14.7%, respectively) (P<0.001 for both comparisons). Conversely, arthralgia was more common in chikungunya (94.9%) and FLAV/CHIKV (100.0%) than in dengue (59.4%) and Zika (53.8%) (P<0.001). A correct presumptive clinical diagnosis was made for 9-23% of the confirmed patients.

Conclusions. Arboviral infections are frequent causes of febrile illness. Co-infections are not rare events during periods of intense, concomitant arboviral transmission. Given the challenge to clinically distinguish these infections, there is an urgent need for rapid, point-of-care, multiplex diagnostics.

INTRODUCTION

Dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) viruses are widely distributed arboviruses, affecting tropical and sub-tropical areas [1]. In Brazil, one of the leading countries in numbers of reported dengue, chikungunya and Zika cases [2], DENV was re-introduced in 1981 [3], and since has been transmitted endemically, with periodic epidemics. The four DENV serotypes have circulated in the country concomitantly since 2010 [4]. In September 2014, the first autochthonous cases of chikungunya were reported in Brazil, almost simultaneously in the north of the country (Amapá state) and in the northeastern region (Bahia state) [5,6]. Less than one year later, early in 2015, autochthonous cases of Zika were first detected in the northeastern states of Bahia and Rio Grande do Norte [7,8]. As CHIKV and ZIKV quickly spread, simultaneous co-circulation of DENV, CHIKV, and ZIKV was established in Brazil and other South and Latin American countries.

Patients infected by DENV, CHIKV or ZIKV may develop an acute illness, with similar clinical characteristics [9]. Symptoms and signs commonly observed include fever, rash, muscle pain, arthralgia, and headache [9]. Due to the difficult in correctly diagnosing these infections based on clinical impressions, laboratory tests are needed for accurate diagnosis in areas of co-circulation. Furthermore, only diagnostic methods can detect concomitant arboviral infections, which may commonly occur during concurrent epidemics and might have important implications for clinical outcomes. However, effective laboratory services are not readily available in most ambulatory and emergency units of tropical and subtropical countries [10]. Thus, few studies have systematically evaluated the frequency of arboviral infections and compared the clinical and epidemiological characteristics of single versus dual infections in settings with co-circulation of these arboviruses.

Herein, we describe results from surveillance designed to monitor arboviral infections among acute febrile patients in Salvador, the capital of the Bahia state, northeastern Brazil, between 2014 and 2016, a period when CHIKV and ZIKV were introduced and spread throughout the country. We present clinical and epidemiological characteristics of laboratory-confirmed cases and, to determine whether human co-infections are more likely acquired through single bites of co-infected mosquitoes or multiple bites by mosquitoes carrying individual arboviruses, we also evaluate whether co-infections were more frequent than would be expected by chance based on the assumption of independent transmission

METHODS

Study design and participants enrollment

From September 2014 to July 2016, we conducted enhanced surveillance to detect DENV, CHIKV, and ZIKV infections among febrile patients attending a public emergency health unit (São Marcos Emergency Center – SMEC), located in the Pau da Lima neighborhood, an urban slum community of Salvador. According to the 2010 National Census, Salvador had 2.5 million inhabitants [11], with 76,352 (3%) of them living in Pau da Lima [12,13].

During the study period, from Monday to Friday, between 7:30 am and 4:00 pm, we prospectively enrolled patients ≥6 months of age who sought medical care with a measured temperature ≥37.8°C or with a history of fever in the prior 7 days. The Research Ethics Committees of the Gonçalo Moniz Institute, Oswaldo Cruz Foundation and Yale University approved the study. Before enrollment, written informed consent was obtained from patients ≥18 years of age, or from guardians of patients <18 years of age, and written assent was obtained from patients 7-17 years of age.

Data and blood sample collection

We interviewed the participants or their guardians using a standardized questionnaire that included demographic and clinical data. Medical chart records were reviewed to obtain data on presumptive clinical diagnoses. An acute blood sample was collected at study entry, and patients were invited to return 15 days later for collection of a convalescent sample. A study team visited the homes of patients who were unable to return to the health unit in order to collect their convalescent blood. During the follow-up for convalescent blood collection, a second interview was performed to obtain data on resolution of signs and symptoms.

Arboviral diagnosis

Blood samples were refrigerated at 2-8ºC and transported to the laboratory on the day of collection. Sera obtained by centrifugation were stored at -20ºC and -70ºC for serological and molecular testing, respectively. Acute sera underwent RNA extraction (Maxwell® Viral Total Nucleic Acid K), followed by reverse transcription-polymerase chain reaction (RT-PCR) for DENV [14], ZIKV [15], and CHIKV [16] with the AccessQuick™ RT-PCR System kit (Promega, USA).

In addition, we performed DENV and CHIKV IgM-ELISA (Panbio Diagnostics, Brisbane, Australia and Inbios, Washington, USA, respectively) on both acute and convalescent sera and tested the former with a DENV NS1-ELISA (Panbio Diagnostics, Brisbane, Australia). We did not employ a ZIKV serological assay due to the low accuracy of the available tests [17,18].

We defined acute DENV, CHIKV, and ZIKV infections by a positive result in the DENV RT-PCR or NS1-ELISA; a positive result in the CHIKV RT-PCR, or seroconversion between

acute and convalescent CHIKV IgM-ELISA; and a positive ZIKV RT-PCR, respectively. Due to potential cross-reactivity in the DENV IgM-ELISA following a ZIKV infection, we classified patients presenting DENV IgM-ELISA seroconversion between acute and convalescent samples and negative RT-PCR results for both DENV and ZIKV as acute flavivirus (FLAV) infections. Patients fulfilling the acute infection case definition for more than one arbovirus were classified as an arboviral co-infection. Finally, because a positive IgM-ELISA in the acute sample may represent a previous, recent and not an acute infection in a context of intense arboviral transmission, we defined patients with a positive DENV or CHIKV IgM-ELISA in the acute sample (or in the convalescent sample when no acute sample was available) as cases of recent undetermined FLAV infection and of recent CHIKV infection, respectively.

Statistical analyses

We calculated the overall frequency for acute and recent arboviral infections, specific frequencies of acute DENV, CHIKV, ZIKV and FLAV infections, and of co-infections, for the whole study period and monthly. Based on the detected frequencies for each virus and the assumption of independent transmission, we estimated the expected frequency of arboviral co-infections and compared them to the observed co-infection frequencies. Demographics, clinical manifestations, and presumptive clinical diagnoses were compared among patients according to their acute infection confirmation status. Medians and interquartile ranges (IQR), or absolute and relative frequencies were used for comparisons. Two-tailed Wilcoxon-Mann-Whitney, Pearson Chi-square, or Fisher exact tests were used as applicable to assess statistical difference between the groups at a $P<0.05$ significance level. Data were collected and stored using the Research Electronic Data Capture (REDCap) software [19]. Statistical analyses were performed using STATA 12 [20].

RESULTS

Laboratory diagnosis of arboviral infection

During the study, we enrolled 948 acute febrile illness patients with at least one sample available for laboratory testing. Both acute and convalescent samples were collected from 428 (45.1%) of the patients, only acute sample from 510 (53.8%), and only convalescent sample from 10 (1.1%). Due to insufficient volumes of sera, RT-PCR for DENV and CHIKV was performed for 915 (96.5%) of the patients and RT-PCR for ZIKV was performed for 914 (96.4%). DENV serological tests were performed for 940 (99.2%) of the patients (45 tested only by IgM-ELISA, 1 tested only by NS1-ELISA, and 894 tested by both), and CHIKV IgM-ELISA was performed for 919 (96.9%).

Of 948 participants, 247 (26.1%) had evidence of an acute arboviral infection, of which 224 (23.6%) were single infections and 23 (2.4%) co-infections (Figure 1). Specifically, 32 (3.4%) patients tested positive for DENV, 159 (16.7%) for CHIKV, 13 (1.4%) for ZIKV, 20 (2.1) for FLAV, 13 (1.4%) for DENV/CHIKV co-infection, 9 (0.9%) for CHIK/FLAV co-infection, and 1 (0.1%) for DENV/ZIKV co-infection (Figure 1). Of the 45 patients with a positive DENV RT-PCR test, 5 (11.1%) were DENV-1, 17 (37.8%) were DENV-3, and 23 (51.1%) were DENV-4.

Based on the observed frequency of DENV, CHIKV, ZIKV, and FLAV infection, the expected frequency of DENV/CHIKV co-infections was 0.9% (~9 cases), CHIKV/FLAV was 0.6 % (~6 cases), DENV/ZIKV was 0.1% (~1 case), and CHIKV/ZIKV was 0.3% (~3 cases). These co-infection frequencies were not statistically different from expected ($P>0.05$ for all the comparisons).

Of the 247 acute arboviral infections, 39 (4.1) had concomitant laboratory evidence for a recent infection, including 4 (0.4%) recent CHIKV among the 32 acute dengue cases, 28 (2.9%) recent FLAV among the 159 acute chikungunya cases, and one recent CHIKV in the sole acute DENV/ZIKV co-infection (Figure 1). In addition, 133 (14.0) other patients without an acute arboviral infection had laboratory evidence for a recent arboviral infection, including 54 (5.7%) with recent CHIKV infection, 60 (6.3%) with recent FLAV infection, and 19 (2.0) with a dual recent CHIKV/FLAV infections (Figure 1).

Clinical manifestations

The median age of acute Zika patients (20 years; IQR: 15-38) was lower than that of acute dengue (30; IQR: 15-38), chikungunya (32; IQR: 20-43), and FLAV patients (35; IQR: 24-42), as well as of DENV/CHIKV (34; IQR: 19-34), and CHIKV/FLAV (47; IQR: 37-51) co-infected patients (P<0.001) (Table 1). The median number of days between fever onset and study enrollment was higher for dengue (4; IQR: 3-4) and lower for chikugunya (1; IQR: 1-3), compared to Zika (2; IQR: 2-3), FLAV infections (3; IQR: 1.5-5), and DENV/CHIKV and CHIKV/FLAV co-infections (2; IQR: 1-2, for both) (P< 0.001) (Table 1).

Headache and myalgia were the most commonly reported symptoms, occurring in >80% of the arboviral patients, as well as among those with a non-arboviral febrile illness. Rash was reported more frequently by patients infected with ZIKV (69.2%), FLAV (55.0%), and DENV/CHIKV (53.8%), compared to those with DENV (37.5%), CHIKV (22.9%), CHIKV/FLAV (11.1%), and those negative for an acute or recent arboviral infection (32.9%) (P<0.001) (Table 1); pruritus followed a similar reporting pattern. Conversely, arthralgia was more frequently reported by patients with CHIKV (94.9%), DENV/CHIKV (84.6%), and

FLAV/CHIKV (100.0%), compared to those with DENV (59.4%), ZIKV (53.8%), FLAV (75.0 %) and non-arboviral patients (62.3%) (P<0.001). Swollen joints were more commonly reported by patients with DENV/CHIKV (53.8%), followed by CHIKV/FLAV (44.4%), FLAV (40.0%), CHIKV (39.6), DENV (31.2%), and ZIKV (30.7%) infections, and much less frequent among non-arboviral patients (17.6%) (P <0.001). The sole patient with evidence for an acute DENV/ZIKV co-infection reported headache, myalgia, retro-orbital pain, rash, pruritus, arthralgia, vomiting and swollen joints.

Nearly all (81 of 86; 94.2%) chikungunya patients who provided a convalescent blood sample remained arthralgic (median 18 days after symptoms onset; IQR: 13-32), as did 100% (4 of 4) of patients with DENV/CHIKV co-infection (median 32 days after onset; IQR: 17-56), and 100.0% (9 of 9) of the followed of patients with CHIKV/FLAV co-infection (median 15 days after onset; IQR: 13-18). In comparison, 56.2% (9 of 16) of the followed dengue patients, 57.1% (4 of 7) of the followed Zika patients, and 65.0% (13 of 20) of the followed FLAV-infected patients maintained arthralgia (P<0.001), with median follow-up of 21 [IQR: 16-44], 30 [IQR: 20-44] and 27 [IQR: 16-46] days after onset, respectively.

Presumptive diagnoses

Despite some differences in clinical manifestations, the accuracy of presumptive diagnosis was poor (Table 1). Among patients with acute DENV infection, only 9.4% were accurately diagnosed, while 18.7% were suspected for ZIKV and none for CHIKV. Among patients with acute CHIKV infection, the most common presumptive diagnosis was DENV (30.8%); a much smaller proportion was suspected of CHIKV (10.7%) or ZIKV (6.9%). A poor pattern of clinical diagnosis was also observed for patients with acute DENV/CHIKV co-infection, with 23.1% suspected as DENV and none suspected as CHIKV; interestingly, 15.4% were

suspected of ZIKV infection. Among those with acute ZIKV infection, 23.1% were correctly diagnosed, while 7.7% were suspected as DENV and none as CHIKV.

Temporal distribution of arboviral infections

Acute arboviral infections were detected through most of the study period, except for November 2014 to March 2015 (Figure 2). Cases of acute DENV, CHIKV, and FLAV infections were confirmed from the first study month (September 2014), whereas acute ZIKV infections were only confirmed in May and July, 2015. Cases of acute DENV infection were mainly detected between April and October 2015, while CHIKV infections peaked between June and November 2015. Consequently, DENV/CHIKV co-infections were mainly found between June and September 2015 and DENV/ZIKV co-infections were only found in July 2015. Of note, CHIKV infections continued to be detected until the last study month, in July 2016 (Figure 2).

DISCUSSION

Our results confirmed the simultaneous transmission of DENV, CHIKV, and ZIKV in northeastern Brazil and revealed the large impact of these viruses as causes of febrile illness requiring medical care. During the study period, 26.1% of the enrolled patients were laboratory-confirmed with an acute arboviral infection. However, in the second semester of 2015, when transmission of CHIKV and DENV peaked, the frequency of any arboviral infection was >50%.

Particularly noteworthy was our finding of CHIKV circulation in Salvador at the same time (September 2014) that it was first detected causing outbreaks in other Brazilian cities [5,6], though apparently major amplification in Salvador only began in June 2015, one month after

the ZIKV epidemic peak in May 2015 [21]. Curiously, ZIKV spread in Salvador was very rapid and the outbreak, comprising ~17,500 case reports, lasted only two months [21,22], while the CHIKV emergence was less abrupt and lasted longer, hampering its prompt recognition, especially because public health attention was directed to the ZIKV outbreak [23]. Although our surveillance study included only one health unit of Salvador, our arboviral detection over time reflected previous citywide observations [21–23].

It remains unclear why ZIKV and CHIKV had different spread patterns in Salvador, both being transmitted mainly by the same *Aedes* (*Stegomyia*) *aegypti* mosquitoes and with the local population entirely susceptible to both. Furthermore, Salvador *Ae. aegypti* are not particularly susceptible to an American strain of ZIKV tested experimentally [24], inconsistent with the explosive amplification that was observed citywide [21]. It is possible that particularities in the interaction between viruses, vectors, and the human population produced different outcomes in terms of vectorial capacity. These may include virus strains variation, human and mosquito' co-infections, human genetic diversity, and variation in the sequence and timing of human arboviral infections.

We also found that co-infections were relatively common, affecting 23 (10.3% of the 224 acute arboviral infections detected and 2.4% of all the febrile patients studied). In addition, 38 (17.0%) of the 224 acute single arboviral infections and 133 (20.0%) of the 701 patients without an acute arboviral infection had laboratory evidence for a recent arboviral infection. These impressively high frequencies of concomitant and sequential infections were apparently only due to the intense simultaneous transmission of the three arboviruses in Salvador during the study period.

Our finding, that the observed frequencies of co-infections did not differ from expected based in independent transmission, suggests that co-infections mainly resulted from sequential bites from mosquitoes infected with different arboviruses, rather from single bites from multiply-infected mosquitoes. Although *A. aegypti* is susceptible to dual infection by the three viruses [25], our data suggest that these might be uncommon in nature. Further studies are needed to investigate this hypothesis and also to better determine whether the pathogenesis and clinical outcome from co-infections and sequential infections differ from those of single infections.

Among the arboviruses we studied, ZIKV presented the lowest frequency. This may be explained by our inclusion criteria requiring the presence of fever, which is less common in ZIKV infections [26]. Second, the sensitivity of ZIKV molecular diagnosis is limited [27], hampering case detection during the viremic phase of the infection, and we did not employ ZIKV serological tests due to their low accuracy [17,18]. Finally, some patients diagnosed with an acute FLAV infection based on DENV IgM-ELISA seroconversion might actually have reflected a ZIKV infection that cross-reacted with DENV.

Interestingly, Zika patients had a lower median age compared other arboviral-infected patients. As ZIKV infections produce milder clinical manifestations, it is possible that ZIKV-infected children were more likely to be brought by their parents or guardians for medical care than adults. It is also possible that Zika clinical manifestations in adults are less prominent than in children. If so, previous DENV exposures, which increase with age, may play an immunolomodulatory role in this difference [28,29].

As previously noticed, we also detected clinical manifestation differences between DENV, CHIKV, and ZIKV infections. Zika patients more frequently had rash and pruritus, as shown

in Brazil [30] and Nicaragua [9], while arthralgia were more common in chikungunya patients, as reported in Trinidad [31]. However, rash and pruritus were also common among non-ZIKV patients, affecting those with DENV and CHIKV, as well as patients with non-arboviral illness. Arthralgia was also very frequent among non-CHIKV patients, occurring in >50% of DENV, ZIKV, and non-arboviral patients. As a caveat, our signs and symptoms data were based on patients' self-reports rather than medical evaluations. Thus, imprecision for some signs, such as joint edema may have occurred. In addition, the generalizability of our findings are limited to febrile patients and do not totally apply to ZIKV-infected patients, who frequently have no detectible fever.

Despite some clinical differences, an erroneous presumptive diagnosis was the rule. Dengue was suspected for <10% of the patients with confirmed DENV single infection, but was suspected for ~30% and ~20% of patients with confirmed CHIKV and DENV/CHIKV infections, respectively. Chikungunya was suspected for ~10% of patients with CHIKV infection and for none with DENV/CHIKV co-infection. These findings may be explained by the lack of physicians' awareness regarding high levels of CHIKV transmission in Salvador [23]. They also suggest that differences between clinical manifestations of DENV and CHIKV infection (possibly related to the severity of symptoms) made physicians suspect dengue two-to-three times more often in patients with confirmed CHIKV infections, compared to patients with confirmed DENV infections.

In summary, our study, conducted in a period of intense, simultaneous DENV, CHIKV and ZIKV transmission, highlights the burden of arboviral diseases for febrile illness and indicates that co-infections are common in these circumstances. Given the clinical similarities among arboviral diseases and the challenge of an accurate clinical suspicion, epidemiological

information on seasonality, population susceptibility, and transmission intensity is needed to improve the accuracy of presumptive clinical diagnoses. However, only with accurate diagnostic tools readily available in local health units, will we be able to provide proper detection, clinical care, and surveillance of arboviral diseases.

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Conflict of interest: SCW owns intellectual property related to chikungunya vaccine development

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Table 1. Clinical characteristics of the febrile illness patients enrolled in the study according to laboratory diagnosis of acute arboviral infection. Salvador, Bahia, Brazil. September 2014-July 2016.

Characteristics	DENV (N: 32)	CHIKV (N: 159)	ZIKV (N: 13)	FLAV (N: 20)	DENV/CHIKV (N: 13)	CHIKV/FLAV (N: 9)	Negative (N: 568)
	Number of cases (%) or median (interquartile range) [a]						
Demographics							
Age [b]	30 (15-38)	32 (20-43)	20 (15-38)	35 (24-42)	34 (19-34)	47 (37-51)	26 (15-37)
Female sex	15 (46.9)	78 (49.1)	7 (53.8)	11 (55.0)	10 (76.9)	3 (33.3)	266 (47.1)
Clinical manifestations							
Days of fever [b]	4 (3-4)	1 (1-3)	2 (2-3)	3 (1.5-5)	2 (1-2)	2 (1-2)	2 (1-4)
Headache	29 (93.5)	148 (93.1)	12 (92.3)	20 (100.0)	12 (92.3)	8 (88.9)	504 (89.2)
Myalgia [b]	25 (80.6)	150 (94.3)	11 (84.6)	17 (85.0)	11 (84.6)	9 (100.0)	452 (80.4)
Retro-orbital pain	20 (64.5)	116 (73.4)	9 (69.2)	15 (75.0)	7 (53.8)	5 (55.6)	348 (62.2)
Arthralgia [b]	19 (59.4)	151 (94.9)	7 (53.8)	15 (75.0)	11 (84.6)	9 (100.0)	354 (62.3)
Swollen joints [b]	10 (31.2)	63 (39.6)	4 (30.7)	8 (40.0)	7 (53.8)	4 (44.4)	100 (17.6)
Vomit	8 (25.0)	36 (22.8)	1 (7.7)	5 (25.0)	6 (46.1)	0	567 (29.8)
Rash [b]	12 (37.5)	36 (22.9)	9 (69.2)	11 (55.0)	7 (53.8)	1 (11.1)	186 (32.9)
Pruritus [b]	10 (31.2)	23 (14.7)	9 (69.2)	10 (50.0)	7 (53.8)	0	196 (34.5)
Presumptive diagnosis recorded on the chart [c]							
Dengue [b]	3 (9.4)	49 (30.8)	1 (7.7)	2 (10.0)	3 (23.1)	2 (22.2)	60 (10.7)
Zika [b]	6 (18.7)	11 (6.9)	3 (23.1)	3 (15.0)	2 (15.4)	1 (11.1)	34 (6.1)
Chikungunya [b]	0	17 (10.7)	0 (0)	0	0	0	8 (1.4)
UVI	3 (9.4)	41 (25.8)	1 (7.7)	4 (20.0)	1 (7.7)	2 (22.2)	87 (15.6)
URI [b,d]	2 (6.2)	3 (1.9)	0 (0)	0	0	0	45 (8.1)
Gastroenteritis	1 (3.1)	0 (0)	0 (0)	0	0	0	13 (2.3)
Cystitis	1 (3.1)	2 (1.3)	0 (0)	1 (5.0)	0	0	5 (0.9)
Other [e]	3 (9.3)	2 (1.3)	1 (7.7)	1 (5.0)	0	0	24 (4.3)
None	14 (43.7)	61 (38.6)	7 (53.8)	11 (55.0)	8 (61.5)	4 (44.4)	309 (55.4)

Note. Of the 948 study patients, data was not shown for one patient with an acute DENV and ZIKV co-infection and for 133 patients with laboratory evidence of recent arboviral infections.

CHIKV= Chikungunya virus; DENV= Dengue virus; FLAV= Flavivirus; UVI = Unspecific viral infection; URI = Upper respiratory infection ZIKV= Zika virus.

[a] Data were not available for some variables: sex, headache, and rash (4 patients each), myalgia (7 patient), retro-orbital pain (11 patients), vomit (2 patients), medical suspicious recorded on the chart (14 patients).

[b] Differences between groups were statistically significant ($P<0.05$).

[c] Sum may be greater than 100% because some patients had more than one clinical impression recorded on the chart.

[d] URI included pharyngitis, sinusitis, and influenza.

[e] Other medical suspicious were leptospirosis, pneumonia, skin infection, rotavirus, viral myositis, appendicitis, HIV, mumps.

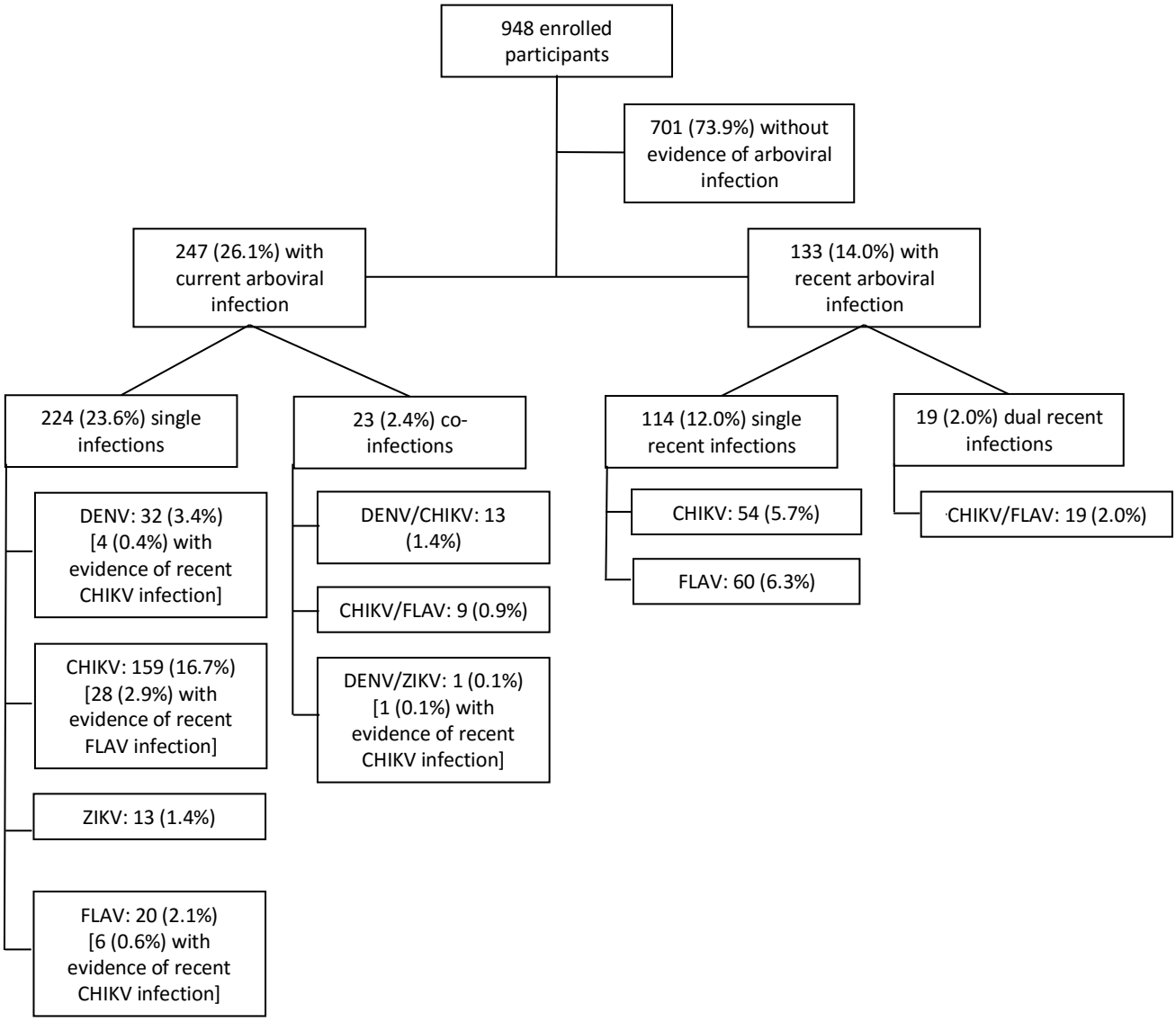

Figure 1. Flowchart of 948 patients enrolled during an acute febrile illness surveillance study in an emergency health unit, according to the arboviral diagnosis, Salvador, Brazil, September 2014 to July 2016.

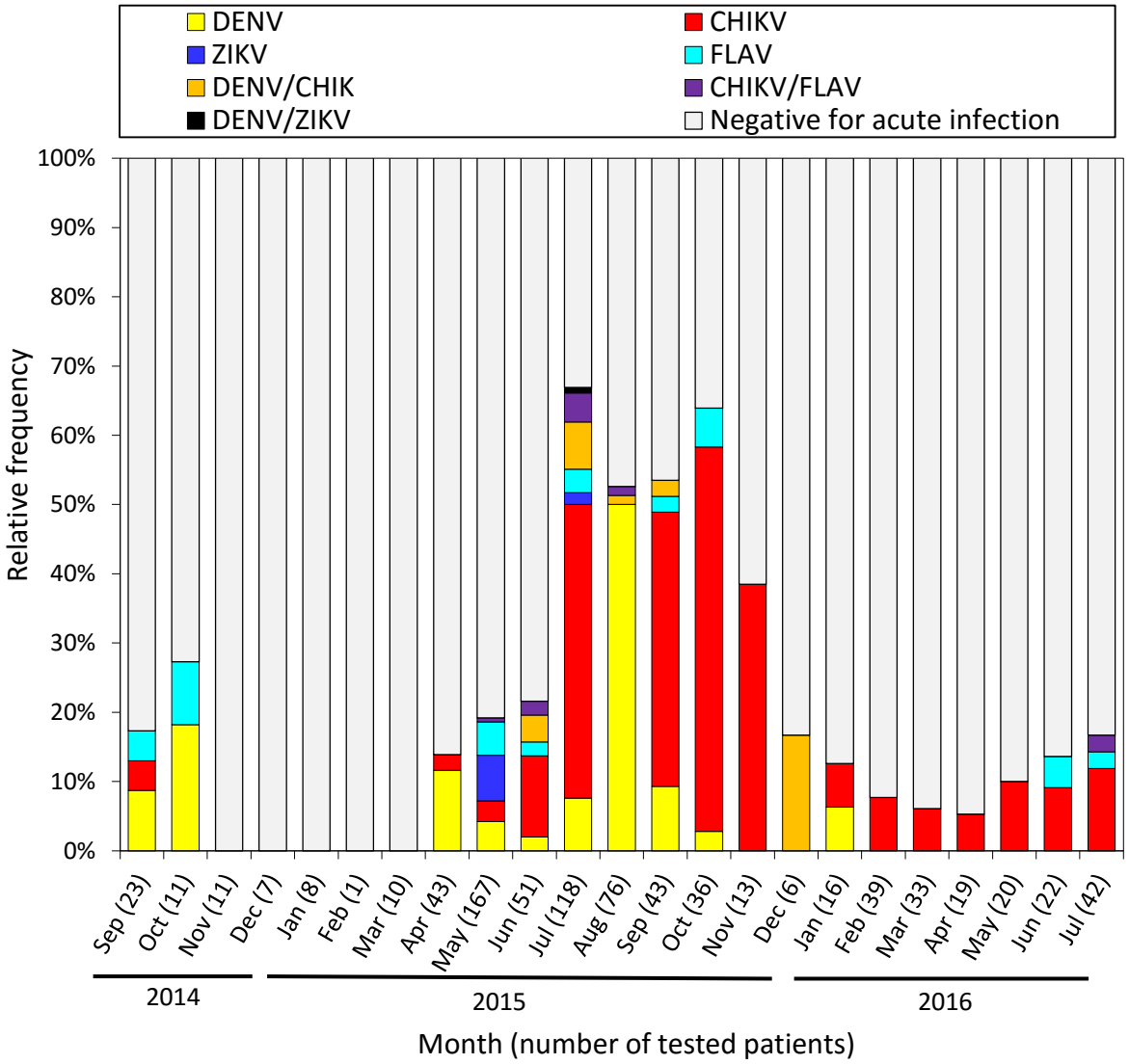

Figure 2. Percent distribution of 948 acute febrile illness patients according to the arboviral diagnosis by month, Salvador, Brazil, September 2014 to July 2016.

3.2 MANUSCRITO 2- Risk of chronic arthralgia in a cohort of patients with laboratory-confirmed chikungunya virus infection in Brazil.

O manuscrito 2 está finalizado em fase de submissão e corresponde ao objetivo específico da tese:

- Estimar o risco de artralgia crônica em pacientes com confirmação laboratorial de infecção por CHIKV, e avaliar os fatores de risco associados.

Risk of chronic arthralgia in a cohort of patients with laboratory-confirmed chikungunya virus infection in Brazil

Running title: Chronic arthralgia following CHIKV infection

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Summary. 42.5% of patients with acute CHIKV infection progressed to develop chronic arthralgia and 30.7% remained symptomatic on average 1.5 years later. 93.8% of them reported limitations to perform daily activities. Female sex and age was found to increase the risk for chronic arthralgia. Our findings reinforce the catastrophic scenario that large CHIKV epidemics may produce in Brazil and elsewhere.

ABSTRACT

Introduction*:* In September 2014, the first autochthonous cases of chikungunya virus (CHIKV) infection were detected in Brazil. The virus rapidly spread and, by April 2018, more than 500,000 cases had been reported by the Ministry of Health. However, the risk of affected patients to evolve to chronic joint pain remains unknown in Brazil.

Aim: To estimate the risk for chronic arthralgia in a cohort of laboratory-confirmed CHIKV infected patients and to assess associated factors.

Methods: From September 2014 to July 2016, a surveillance study performed in a health emergency center of Salvador, Brazil detected laboratory-confirmed CHIKV infection cases by IgM ELISA or RT-PCR. During study enrollment, the surveillance team interviewed the patients to collect data on socio-demographics and initial clinical manifestations. Telephone follow-up was performed on average 1.5 years after case detection to obtain data on disease evolution.

Results: From the 948 febrile patients tested to CHIKV infection, 265 (27.9%) were laboratory confirmed. Of them, we were able to follow 153 (57.7%) and 65 (42.5%) reported having chronic arthralgia that lasted >3 months; 47 (30.7%) were still symptomatic. The chronic joint pain was more common in the fists (95.4%), ankles (92.3%), knees and fingers (90.7%). Joint edema was reported by 76.9% of the patients with chronic arthralgia. The intensity of the pain was classified as severe by 66.1% of the patients and 93.8% of them reported limitations to perform daily activities. Female sex and age was found to increase the risk for chronic arthralgia.

Conclusion: Our findings reinforce the high frequency of chronic arthralgia following CHIKKV infection and its related disabilities. Development of novel strategies to mitigate CHIKV transmission, as well as to provide long-term medical assistance for infected patients are critically needed.

Key words: Chikungunya, Arthritis, Arthralgia, Chronic manifestations, Chronification, Epidemiology.

INTRODUCTION

Chikungunya virus (CHIKV) is an arbovirus of the genus Alphavirus, family Togaviridae, transmited by *Aedes aegypti* and/or *Aedes albopictus* mosquitoes [1]. The first human cases of CHIKV infection were identified in the province of Newala, Tanzania in 1952 [2]. Since then, CHIKV was associated with numerous outbreaks in Africa [3,4] and Asia [5,6]. In 2013, the first autochthonous transmission was reported in the Americas [7,8]. In Brazil, CHIKV transmission was first detected in September 2014 in two cities located ~3,300 km apart (Oiapoque, in the north state of Amapá, and Feira de Santana, in the northeast state of Bahia) [9,10].

Typical clinical manifestations of CHIKV infection include fever of abrupt onset, headache, backache, and arthralgia, which is the hallmark symptom [11]. The clinical picture is didactically divided into three stages: acute, subacute and chronic [12]. The acute stage lasts for about ten days and is characterized by the presence of fever and polyarthralgia, but other manifestations, such as headache, rash, conjunctivitis, nausea, abdominal pains, and joint edema are commonly observed. During this stage, the symptoms may subside or evolve to a subacute stage characterized by maintenance of the arthralgia. If the arthralgia does not remit, the disease enters the chronic stage, which is defined by the persistence of arthralgia and other unspecific symptoms for periods greater than three months, eventually reaching three or more years [13,14]. In this stage, arthralgia typically affects multiple joints, in a relapsing-remitting or persistent pattern.

Chronic arthralgia was estimated to affect up to 60% of the CHIKV infected patients [15] causing important disabilities for the everyday activities [12,14]. During this stage,

the patient may present both physical and mental suffering, reporting inappetence, non-restorative sleep, labor and daily activities impairment, mood swings, and depression. Such symptoms can cause employment and school withdrawal, as well as compromise the quality of life [13,15]. Herein, we present findings from the follow-up of a cohort of CHIKV infected patients in order to characterize their clinical manifestations, estimate the frequency in which they evolved to chronic arthralgia, asses risk factors for joint pain persistence, and evaluate the impact of the disease in patients' daily activities.

METHODS

Study design and participants selection

The cohort of patients with laboratory-confirmed CHIKV infection was established between September 2014 and July 2016, during an enhanced surveillance study to detect arboviral infections among acute febrile illness patients searching for medical care. Surveillance enrollment was performed from Monday to Friday, from 7:30 am to 4 pm, at a public emergency health unit, located in the neighborhood of Pau da Lima, Salvador, Bahia, Brazil. Inclusion criteria were age ≥6 months, residence at Salvador, and reported or measured fever (temperature ≥37.8°) lasting ≤ 7 days.

During surveillance enrollment, we interviewed the patients to obtain data on socio-demographics, clinical manifestations, and contact information for subsequent follow-up. In addition we collected a blood sample for arboviral diagnostic tests. We invited the enrolled patients to return to the health unit 15 days later to collect a convalescent-phase blood sample. A study team visited the houses of the patients who lived within Pau da Lima neighborhood and did not return for the convalescent-phase blood sample collection in order to collect the second blood sample. We also reviewed medical charts

to obtain data on clinical impression, laboratory exams eventually performed, medical care received, and discharge or hospitalization.

Laboratory diagnosis

The blood samples collected were kept refrigerated at 2-8ºC and daily transported in cool boxes to the Laboratory of Pathology and Molecular Biology, Gonçalo Moniz Institute, Oswaldo Cruz Foundation, located ~15km far from the surveillance health unit. There, the blood samples were centrifuged and the sera were aliquoted and stored at -20ºC and -70ºC until the diagnostic experiments. Acute-phase serum samples underwent RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) for CHIKV [16]. Acute- and convalescent-phase samples were tested by enzyme-linked immunosorbent assay (ELISA) for the presence of IgM antibodies against CHIKV (Inbios, Washington, EUA). We defined a patient with positive results in any of the above mentioned tests as a laboratory-confirmed case of CHIKV infection. Patients were also tested for dengue (DENV) and Zika (ZIKV) viruses infection by RT-PCR [17,18]. Serological methods (IgM and NS1 ELISA; Panbio Diagnostics, Brisbane, Australia) were used to aid detection of DENV infections.

Cohort follow-up

Between November/2016 and March//2017, on average 1.5 years (SD: 0.3) after symptoms onset, we attempted telephone contact with the laboratory-confirmed cases of CHIKV infection in order to interview them about disease progression. Contacts were attempted from Monday to Sunday, from 8 am to 9 pm. The minimum number of times that we attempted to talk with each patient was three. Patients who we could not contact

due to moving to another city, incorrect or unavailable telephone number, or who refused to be interviewed were considered loss to follow-up.

During the interview, a standardized questionnaire was used to collect data on the evolution of clinical manifestations. The main focus of the interview was to determine whether the artralgia persisted or subsided, as well as its duration. We considered patients who reported persistent joint pain for more than three months as those with chronic chikungunya. Such patients had additional data collected to characterize the affected joints, pain intensity, presence of other clinical manifestations, mental distress, as well as the disease impact in regular daily activities, such as maintenance of work and/or studies, and the demand for health care. The pain intensity was assessed by a verbal pain score scale measured from 0 to 10, which was then classified as mild pain (0-2 in the scale), moderate pain (3-7 in the scale), and severe pain (8-10 in the scale) [19,20]. Mental distress was assessed by the twenty questions present in the Self-Reporting Questionnaire (SRQ-20). This screening instrument used to detect common mental disorders had been previously applied and validated in Brazil [21–23]. Patients with a sum of seven or more affirmative responses in the SRQ-20 regarding the period of persistent arthralgia were considered as individuals presenting mental distress.

Data analysis

We graphically showed the monthly frequencies of CHIKV infections among the febrile patients to present the temporal distribution of detected cases during surveillance enrollment. To verify if the loss to follow-up had introduced a substantial bias in our cohort, we compared the frequencies of social and demographic characteristics, as well as of clinical manifestations at surveillance enrolment between the laboratory-confirmed

CHIKV infection cases who completed and who did not complete the telephone follow-up survey. Frequencies of sociodemographic characteristics, clinical manifestations, disease impact on regular daily activities, and medical health demands were used to describe the CHIKV infected patients who evolved with chronic arthralgia. The risk of chronic arthralgia and its 95% confidence interval (95% CI) was calculated by the ratio between the number of CHIKV infected patients who maintained joint pain for >3 months after its onset and the number of patients who completed the telephone follow-up survey. The risk of chronic arthralgia was also calculated by sex, age, acute-phase clinical manifestations of the disease, prior comorbidities, and presence of laboratory evidence of co-infection with DENV during surveillance enrollment. Crude risk ratios and respective 95% CIs for these factors were estimated and those with a chi-square P value ≤0.20 were included in multiple variable Poisson logistic regression analyses with robust variance and backward elimination. Factors associated with chronic arthralgia at a P <0.05 in the multiple variable analysis were considered statistically significant and adjusted risk ratios and 95% CIs were shown. Data were collected and stored in the Research Electronic Data Capture (REDCap) software [24]. Statistical analyses were performed with STATA 12 [25].

Ethical Considerations

Patients who accepted to participate in the study provided written informed consent when aged ≥18 years or written informed assent when aged 7-17 years. Legal guardians also provided written informed consent for inclusion of patients <18 years of age. This study was approved by the Ethics Research Committee of the Gonçalo Moniz Institute, Oswaldo Cruz Foundation.

RESULTS

Clinical characteristics of acute CHIKV infection

Of 948 acute febrile illness patients enrolled in the surveillance study, 265 (27.9%) were laboratory-confirmed as cases of CHIKV infection (Figure 1). Of the confirmed cases, 87 (32.8%) were positive sole by RT-PCR, 114 (43.0%) sole by IgM-ELISA (in either the acute- or the convalescent-phase serum sample), and 64 (24.1%) by both RT-PCR and IgM-ELISA. Except for the period between December 2014 and February 2015, CHIKV infection cases were detected during all the study period, but it occurrence peaked between July and November 2015 (Figure 2).

Of the 265 laboratory-confirmed cases, 133 (50.2%) were females and majority (190; 71.7%) had 15-49 years of age (Table 1). The most common acute-phase clinical manifestations were myalgia (246; 93.5%), headache (245; 92.5%), and arthralgia (234; 88.3%) (Table 1). Other acute-phase manifestations included prostration (230; 87.5%), retro-orbital pain (187; 70.8%), abdominal pain (105; 39.7%), and rash (85; 32.2%). Completion of follow-up was attained for 153 (57.7%) of the confirmed cases of CHIKV infection. Although the cases who completed follow-up were older compared to those who did not complete the follow-up (Table 1), this difference was not statistically significant (P: 0.08). Additional demographics and acute-phase clinical characteristics were similar between the followed and the loss to follow-up cases.

Incidence and clinical characteristics of chronic CHIKV infection

Of the 153 confirmed cases followed, 65 (42.5%; 95% CI: 34.9%-50.4%) reported chronic joint pains that lasted for more than 90 days and 47 (30.7%; 95% CI: 24.0%-38.4%) were still symptomatic at the telephone survey performed on average 1.5 (SD: 0.3) years after onset of the acute illness (Figure 1). Similarly to what was observed for

the whole group of 265 CHIKV confirmed cases, the most frequent acute-phase clinical manifestations among the 65 cases who evolved with chronic disease were arthralgia (65; 95.3%), myalgia (61; 93.8%), and headache (60; 92.3%). The chronic joint pain more frequently affected fists (62; 95.4%), ankles (60; 92.3%), knees (59; 90.7%), and fingers (59; 90.7%). All except by one of these patients classified the joint pain as severe (43; 66.1%) or moderate (21; 32.3%). Joint edema during this chronic disease stage was reported by 50 (76.9%) cases, being more frequent in the toes (38; 58.5%), fingers (36; 55.4%), and ankles (35; 53.8%). (Table 2). Other commonly reported symptoms accompanying the chronic arthralgia were fatigue (57; 87.7%), hair loss (17; 26.1%), and oral ulcers (11; 16.9%).

Risk factors for chronic CHIKV associated arthralgia

Bivariate analysis showed that women had increased risk of developing chronic arthralgia compared to men (RR: 1.91; 95% CI: 1.26-2.88) (Table 3). The risk of chronic arthralgia also increased by age (RR: 1.02; 95% CI: 1.00-1.03 for each one year increase in age). Among the acute-phase clinical manifestations reported during enrollment by the acute febrile illness surveillance, arthralgia was the symptom that best predicted development of chronic arthralgia, although the association was not statistically significant (RR: 2.50; 95% CI: 0.91-7.33). Among the self-reported comorbidities, hypertension was the only prior illness associated with chronic arthralgia (RR: 1.61; 95% CI: 1.10-2.35). Multiple variable analysis found sex (adjusted RR for female: 1.79; IC 95%: 1.95-2.69) and age (RR: 1.02; 95% CI: 1.01-1.03 for each one year increase in age) as the sole independent risk factors associated with development of chronic arthralgia (Table 4).

Self-reported impact of chronic CHIKV infection

When asked about the impact of the chronic joint pain on daily activities, 61 of the 65 (93.8%) patients reported limitations to ordinary activities, such as combing hair, brushing teeth, dressing, and feeding; and 57 (87.7%) of them reported limitation to walk (Table 4). Impact on working activities was perceived by 7 (17.5%) of the 40 patients with chronic disease who worked; 4 (10.0%) reported losing the job and 3 (7.5%) reported having to change the job because of the illness (Table 4). Need of health assistance during the chronic stage of the disease was reported by 33 (50.8%) of the patients. Of the 26 (40.0%) patients who had at least one outpatient appointment with a general physician, 21 (80.8%) were provided by the Brazilian public health system. In contrast, only 7 (10.7%) of the patients reported physiotherapy care, being 3 (42.9%) of them provided by the public health system. Although 7 (10.7%) patients reported the need of hospitalization, only 3 (4.6%) reported receiving health care from a rheumatologist. Of special note, during the chronic stage of the disease, 40 (61.5%) patients presented physical and psycho-emotional symptoms compatible with mental distress, but only 1 (1.5%) reported receiving support from a psychologist.

DISCUSSION

We found that 42.5% of patients with acute CHIKV infection progressed to develop chronic arthralgia and 30.7% remained symptomatic on average 1.5 years later. Arthralgia were considered severe by majority (66%) of those who evolved with chronic joint pain and more than 90% of them reported limitations to perform daily activities. Increase in age and female sex were associated with higher risk of chronic arthralgia.

Our data reinforces' that persistent joint pain is frequent in patients after chikungunya infection and draws attention to the relevance of this problem. Some studies have explored hypotheses that suggest what the cause the persistence of arthralgia is. Researchers identified persistent CHIKV antigens in the synovial fluid of a patient with chronic arthralgia, indicating that the chronicity of arthralgia are linked to local joint inflammation and suggests that viral persistence in the joints may cause chronic arthralgia [26,27]. In addition, experimental studies in CHIKV infected animal indicate that the joint is the most highly infected tissue, making it plausible that incomplete viral antigen clearance in this anatomical site may account for the long-term symptoms [28].

Between December 2007 to June 2008, after the CHIKV outbreak in the Reunion Island, Indian Ocean, CHIKV confirmed patients aging ≥15 years were followed by telephone interviews on average 2 years after the acute stage of the disease and 43.3% reported persistent arthralgia for more than 3 months [29]. High frequencies of long-term persistent arthralgia after CHIKV infection have also been reported in Caribbean [30], France [15,27], Colombia [31] Mexico [32], and in Brazil [33], ranging from 32% to 64%. In such studies, the interval between the acute infection and the patients' follow-up ranged from 6 to 36 months.

The large difference in the time elapsed between the acute disease stage and the patients' follow-up, as well as the subjectivity in pain perception and reporting may partially explain the distinct estimates of risks for development of chronic arthralgia. Alternatively, reported risk differences may result of different genetic backgrounds among these populations and varieties in the circulating CHIKV strain, which may

include antigenic diversity, difference in replication capacity, and distinct potential to induce inflammatory joint pathology [27,34].

In our study, participants reported difficulties to perform daily activities, to walk, and work. These limitations, also documented in other studies [15,27,35,36] shows that chronic arthralgia is highly disabling for daily life activities and professional activities. This issue is especially worrisome because the persistence of joint pain touches people of productive age, affecting individual and collective work such as economic activities, putting pressure on the social security system. Signs of mental distress and/or depressed mood in patients with chronic CHIKV-related pain occurred for 60% of our participants, corroborating previous studies [27,35]. These complications have a significant burden on individual's quality of life, not only because of mental suffering itself, but also due to the difficulty in obtaining public access to specialized care. The early diagnosis of psychic disorders and the follow-up of individuals by specialized professionals are fundamentals for the promotion of mental health. It is recommended that health professionals follow up CHIKV-infected patients until full recovery in order to ensure a return normal health and life quality.

We found age and sex as independent risk factors for chronic arthralgia. Several other studies have also found that older individuals and women have greater risk of maintaining persistent joint pain [27,29,31,32,36]. It is well established that older people have the highest risk of developing rheumatologic diseases [37] and the age-related propensity for rheumatic pain may at least partly explain the association between age and chronic pain observed for CHIKV infection. Moreover, a general decline in both the innate and adaptive immune responses could explain the increased disease

severity observed in older individuals [38]. The greater risk for chronic pain observed among women may be related by psychosocial and biological factors. For example, at a psychosocial level, gender differences in expression of pain are often attributed to the effects of stereotypic sex roles. Pain perception is a complex phenomenon, thus, the role of gender in beliefs and expectations concerning pain may be important in determining the joint pain experience in CHIKV infected subjects [27,39]. From a more biological perspective, hormonal are inevitably associated with and influenced by masculine versus feminine sex roles. Especially in older women, some studies note that hormonal changes can affect the immune system and influence the severity of joint pain [40,41]. However, the mechanisms underlying these sex differences have yet to be fully uncovered.

This study has some limitations. Patient follow-up was performed, on average, 1 year and 5 months after the acute phase of the disease, and participants may not accurately remember the duration of joint pain and signs and symptoms related to CHIKV infection. The loss of 43% of the patients at follow-up was also a limitation to be considered, since it had an impact on the statistical power to identify associations. Another possible limitation is related to laboratory diagnosis because some patients were confirmed by chikungunya virus infection only by detection of IGM antibodies against CHIKV by ELISA, in the first sample collected, suggesting a recent or acute infection. However, in our study, participants are being reported and clinically characterized as patients who had an acute chikungunya infection.

Although it has limitations, this is the first cohort study in Brazil that determines the risk of individuals with laboratory confirmation of CHIKV infection to develop chronic

pain, and is based on the follow-up of patients since the acute phase of the disease. We also investigate the impact of joint pain on patient's daily lives and emphasize that persistent arthralgia, which is often continuous and debilitating, is a burden on individuals' lives. In addition, we identified risk factors associated with chronic arthralgia. Thus, we are confident that the limitations of the study have not change the scope of our results and the relevance of our findings.

In summary, our findings reinforce the catastrophic scenario that large CHIKV epidemics may produce in Brazil and elsewhere, with >40% of the patients presenting a symptomatic acute infection developing chronic arthralgia that may last for about 1.5 years for up to 30% of the patients. The disabling consequences of the persistent joint pain largely influence daily activities and also affect the work capacity, which may have a dramatic impact on the society productive force. Further studies, aiming to determine the role of immunological and genetic factors in the persistence of joint pain, will be critical to improve our understanding of the disease immuno-pathogenesis and to guide novel strategies for patients care. In addition, we urge for the development of CHIKV vaccines, which may definitively prevent future epidemics.

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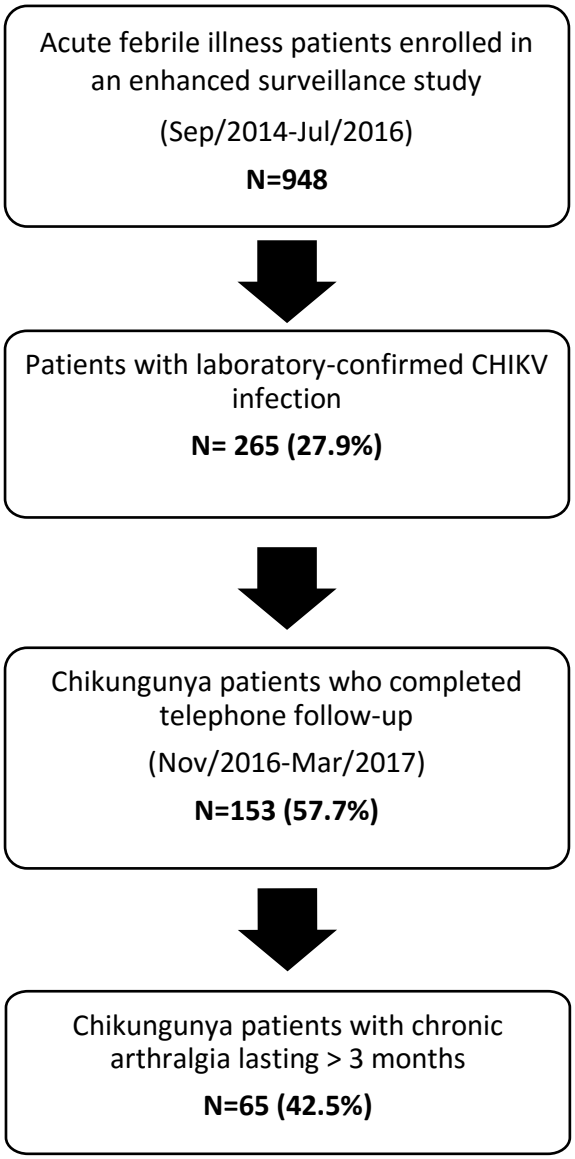

Figure 1. Detection and follow-up of patients with laboratory-confirmed CHIKV infections, Salvador, Brazil.

Table 1. Self-reported enrollment characteristics of 265 patients with laboratory-confirmed CHIKV infection, according to completion of follow-up, Salvador, Brazil, September 2014 – July 2016.

Characteristics	Total patients (N=265)		Patients who completed follow-up			
			Yes (N=153)		No (N=112)	
	No. patients	No. (%)	No. patients	No. (%)	No. patients	No. (%)
Female sex	265	133 (50.2)	153	80 (52.3)	112	53 (47.3)
Mean age, in years (SD)	265	33.9 (16.7)	153	36.2 (17.1)	112	30.9 (15.7)
Age group, in years						
0-14	265	32 (12.1)	153	16 (10.5)	112	16 (14.3)
15-29	265	79 (29.8)	153	38 (24.8)	112	41 (36.6)
30-49	265	111 (41.9)	153	71 (46.4)	112	40 (35.7)
≥50	265	43 (16.2)	153	28 (18.3)	112	15 (13.4)
Skin color/race						
White	265	12 (4.5)	153	6 (3.9)	112	6 (5.4)
Black	265	95 (35.8)	153	61 (39.9)	112	34 (30.4)
Mixed	265	137 (51.7)	153	76 (49.7)	112	61 (54.5)
Yellow	265	13 (4.9)	153	4 (2.6)	112	9 (8.0)
Indigenous	265	8 (3.0)	153	6 (3.9)	112	2 (1.8)
Years of study						
≤5 years of study	258	53 (20.5)	148	30 (20.3)	110	23 (20.9)
6 to 9 years of study	258	81 (31.4)	148	45 (30.4)	110	36 (32.7)
≥ 10 years of study	258	124 (48.0)	148	73 (49.3)	110	51 (46.4)
Occupancy[1]						
Any work	220	153 (69.6)	129	85 (65.9)	91	68 (74.7)
Formal work	220	80 (36.4)	129	47 (36.4)	91	33 (36.3)
Reported clinical manifestations[2]						
Myalgia	263	246 (93.5)	153	141 (92.2)	112	105 (93.7)
Headache	265	245 (92.5)	153	140 (91.5)	112	105 (93.7)
Arthralgia/location	265	234 (88.3)	153	136 (88.8)	112	98 (87.5)
Elbows	260	189 (72.7)	150	110 (73.3)	110	79 (71.8)
Fists	258	202 (78.3)	149	121 (81.2)	109	81 (84.3)
Fingers	261	205 (78.5)	150	117 (78.0)	111	88 (79.3)
Ankles	251	190 (75.7)	145	110 (75.9)	106	80 (75.5)
Knees	262	204 (77.8)	150	120 (81.3)	112	84 (75.0)
Prostration	263	230 (87.5)	151	132 (87.4)	112	98 (87.5)
Retro-orbital pain	264	187 (70.8)	153	109 (71.2)	111	78 (70.3)
Abdominal Pain	264	105 (39.7)	153	60 (39.2)	111	45 (40.5)
Rash	263	85 (32.2)	151	46 (30.4)	112	39 (34.8)
Sore throat	262	74 (28.3)	153	41 (26.8)	109	33 (30.3)
Pruritus	265	70 (26.4)	153	40 (26.1)	112	30 (26.8)
Vomit	264	63 (23.9)	152	33 (21.7)	112	30 (26.8)

[1] Data on occupancy was analyzed for participants aged ≥18 years.
[2] Frequency of clinical manifestations may have been sub-estimated because the two children <3 years of age may have not reported some symptoms.

Table 2. Self-reported characteristics of 65 patients with laboratory-confirmed chikungunya virus infection and chronic arthralgia lasting >3 months, Salvador, Brazil, November 2016 – March 2017.

Characteristics	No. (%)
Age group (years)	
0-14	3 (4.6)
15-29	10 (15.4)
30-49	38 (58.5)
≥50	14 (21.5)
Clinical Manifestations at enrollment	
Arthralgia	62 (95.3)
Myalgia	61 (93.8)
Headache	60 (92.3)
Prostration	58 (89.2)
Retro-orbital pain	51 (78.5)
Abdominal pain	25 (38.5)
Rash[1]	24 (37.5)
Pruritus	18 (27.7)
Vomit	17 (26.1)
Sore Throat	16 (24.6)
Chronic arthralgia	65 (100)
Location of chronic arthralgia [2]	
Fists	62 (95.4)
Ankles	60 (92.3)
Knees	59 (90.7)
Hand fingers	59 (90.7)
Toes	55 (84.6)
Column	52 (80.0)
Shoulders	51 (78.5)
Elbows	45 (69.3)
Neck	40 (61.5)
Intensity of chronic arthralgia	
Severe	43 (66.1)
Moderate	21 (32.3)
Mild	1 (1.5)
Chronic joint edema	50 (76.9)
Chronic joint edema location	
Toes	38 (58.5)
Hand fingers	36 (55.4)
Ankles	35 (53.8)
Knees	31 (47.7)
Fists	27 (41.5)
Elbows	12 (18.5)
Shoulder	9 (13.8)
Column	3 (4.6)
Neck	1 (1.5)
Other chronic symptoms	
Fatigue	57 (87.7)
Hair loss	17 (26.1)
Oral ulcer	11 (16.9)
Skin vesicles	5 (7.7)

[1] Data available for 64 patients.
[2] Of the 65 patients presenting chronic arthralgia, 62 reported arthralgia at enrollment.

Table 3 – Factors associated with chronic arthralgia in CHIKV infected patients. Salvador, Brazil.

Characteristics	No. CHIKV infected patients followed (N: 153)	No. (%) CHIKV infected patients with chronic arthralgia (N:65)	Crude risk ratio (95% CI)	Adjusted risk ratio (95% CI)*
Sex				
Female	80	44 (55.0)	1.91 (1.26-2.88)	1.79 (1.95-2.69)
Male	73	21 (28.8)	1	1
Age, in years	153	-	1.02 (1.00-1.03)	1.02 (1.01-1.03)
Skin color/race				
Black or mixed	137	60 (43.8)	1.40 (0.66-2.97)	-
Non-black and non- mixed	16	5 (31.2)	1	
Acute-phase Manifestations				
Arthralgia				
Yes	136	62 (45.6)	2.50 (0.91-7.33)	-
No	17	3 (17.6)		
Myalgia				
Yes	141	61 (43.3)	1.29 (0.57-2.95)	-
No	12	4 (33.3)		
Headache				
Yes	140	60 (42.8)	1.11 (0.54-2.27)	-
No	13	5 (38.4)	1	
Prostration				
Yes	132	58 (43.9)	1.19 (0.64-2.21)	-
No	19	7 (36.8)		
Retro-orbital-pain				
Yes	109	51 (46.8)	1.47 (0.91-2.36)	-
No	44	14 (31.8)	1	
Rash				
Yes	46	24 (52.2)	1.36 (0.94-1.98)	-
No	105	40 (38.0)	1	
Vomit				
Yes	33	17 (51.5)	1.27 (0.85-1.89)	-
No	119	48 (40.3)	1	
Pruritus				
Yes	40	18 (45.0)	1.08 (0.72-1.62)	-
No	113	47 (41.6)	1	
Sore Throat				
Yes	41	16 (39.0)	0.89 (0.57-1.38)	-
No	112	49 (43.7)	1	
Abdominal Pain				
Yes	60	25 (41.7)	0.96 (0.66-1.41)	-
No	93	40 (43.0)	1	
Self-reported comorbidities				

Diabetes Mellitus				
Yes	5	2 (40.0)	0.93 (0.31-2.79)	-
No	148	63 (42.8)	1	
Hypertension				
Yes	24	15 (62.5)	1.61 (1.10-2.35)	-
No	129	50 (38.8)	1	
Arthrosis				
Yes	17	10 (58.8)	1.45 (0.93-2.27)	-
No	136	55 (40.7)	1	
Overweight[1]				
Yes	46	20 (43.5)	1.03 (0.69-1.53)	-
No	107	45 (42.0)	1	
Dengue virus co-infection[2]				
Yes	12	5 (41.7)	0.96 (0.48-1.93)	-
No	139	60 (43.12)	1	

[1] Overweight was defined as body mass index (BMI) ≥25kg/m^2. BMI was calculated based on self-reported weight and height.
[2] Dengue virus (DENV) co-infection was detected by a concomitant positive result in DENV RT-PCR. Two patients did not have result RT-PCR.
* Poisson logistic regression analyses with robust variance and backward elimination.
(P≤0.20: Sex, Age, Arthralgia, Rash, Pain-retrorbirtal, HAS, Arthrosis).

Table 4. Self-reported impact on daily life activities, working, demand for health care, and mental distress in 65 chikungunya patients who evolved with chronic arthralgia. Salvador, Brazil.

Self-reported impact	**No. (%)**
Impact on daily life activities	
Limitation to ordinary activities[1]	61 (93.8)
Limitation to walk	57 (87.7)
Impact on working[2]	
Loss of job	4 (10.0)
Needed to change the job	3 (7.5)
Health care need due to chronic chikungunya [3]	
Any kind of health care	33 (50.8)
General outpatient clinic	26 (40.0)
Physiotherapy	7 (10.7)
Rheumatology specialist	3 (4.6)
Psychology specialist	1 (1.5)
Hospitalization	7 (10.7)
Mental distress [4]	40 (61.5)

[1] We considered as ordinary activities combing hair, brushing teeth, dressing, and feeding.
[2] Analyses performed for the 40 patients who reported to work during enrollment.
[3] The health care was provided by the public unified health system, except for 5 general outpatient clinic consultations, 4 physiotherapy consultations, and 2 rheumatologist consultations.
[4] Mental distress was assessed by the standardized and validated SRQ-20 questionnaire [Harding et al, 1980]. Patients who answered "yes" for ≥7 of the 20 questions regarding physical and psycho-emotional symptoms in the period of chronic arthralgia were considered to present mental distress.

Supplementary table 1. Responses to the individual questions of the self-reported questionnaire (SRQ-20) to determine occurrence of mental distress among laboratory-confirmed chikungunya virus infection patients.

Physical and psycho-emotional symptoms assessed by the SRQ20	**No. (%)**
Do you often have headaches?	36 (55.4)
Is your appetite poor?	45 (69.2)
Do you sleep badly?	36 (55.4)
Are you easily frightened?	31 (47.7)
Do your hands shake?	24 (36.9)
Do you feel nervous, tense or worried?	43 (66.1)
Is your digestion poor?	15 (23.1)
Do you have trouble thinking clearly?	19 (29.2)
Do you feel unhappy?	35 (53.8)
Do you cry more than usual?	26 (40.0)
Do you find it difficult to enjoy your daily activities?	32 (49.2)
Do you find it difficult to make decisions?	22 (33.8)
Is your daily work suffering?	23 (35.4)
Are you unable to play a useful part in life?	21 (32.3)
Have you lost interest in things?	24 (36.9)
Do you feel that you are a worthless person?	19 (29.2)
Do you feel tired all the time?	40 (61.5)
Are you easily tired?	38 (58.5)
Has the thought of ending your life been on your mind?	8 (12.3)
Do you have uncomfortable feelings in your stomach?	22 (38.8)
Mental distress[1]	**40 (61.5)**

[1] Mental distress was assessed by the standardized and validated SRQ-20 questionnaire [Harding et al, 1980]. Patients who answered "yes" for ≥7 of the 20 questions regarding physical and psycho-emotional symptoms in the period of chronic arthralgia were considered to present mental distress.

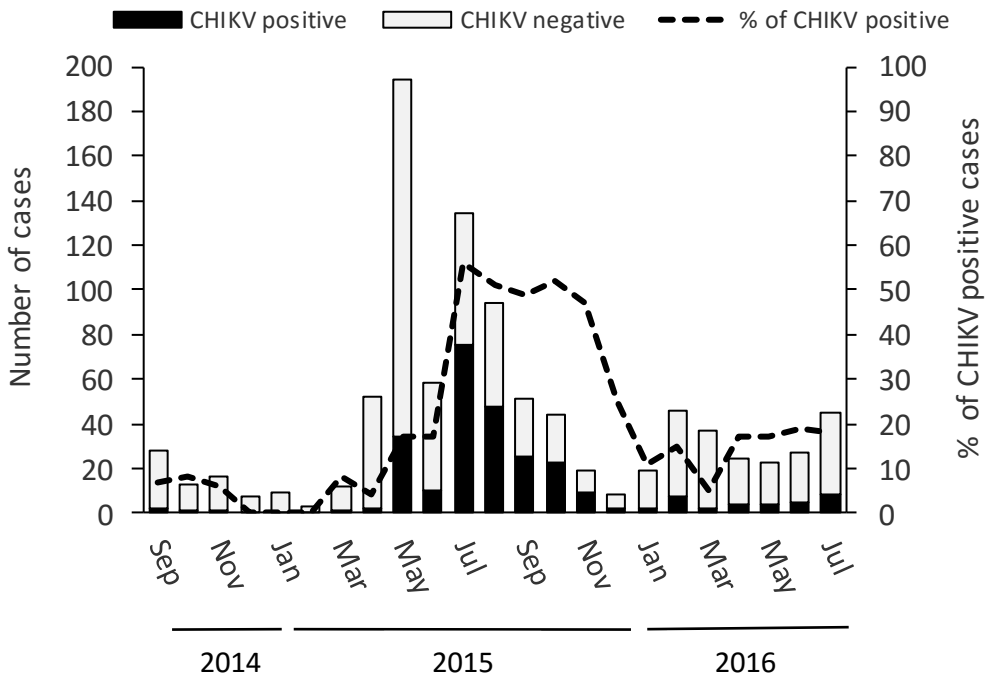

Figure 2 – Distribution of acute febrile illness patients and chikungunya virus laboratory-confirmed cases, according to month and year of case detection. Salvador, Brazil, Brazil, September 2014 to July 2016.

4 DISCUSSÃO

Nossos resultados confirmam a transmissão simultânea do DENV, CHIKV e ZIKV em Salvador, comprova a circulação do CHIKV nesta cidade, desde setembro de 2014, e demonstra evidências de co-infecções, chamando a atenção para a urgência em realizar novos estudos para esclarecer os mecanismos de transmissão dos vírus concomitante e sequenciais, e investigar a influência das co-infecções nas expressões clínicas e gravidade da doença.

Nossos achados reforçam a dificuldade de um diagnóstico laboratorial baseado em testes sorológicos para ZIKV e DENV, devido a semelhanças antigénicas entre eles, possibilitando que anticorpos contra ZIKV reajam de forma cruzada com DENV, em uma área de co-circulação (WAGGONER *et al*, 2016a). Portanto, entende-se a necessidade crescente de um diagnóstico diferencial em pacientes nesse cenário epidemiológico.

Não obstante identificarmos algumas diferenças nas manifestações clínicas entre os pacientes confirmados laboratorialmente por DENV, CHIKV ou ZIKV, em geral a sintomatologia apresentada na fase aguda da doença, pelos pacientes, são similares entre as arboviroses, e comum em infecções por outros patógenos causadores de doenças febris, (REZZA *et al*, 2014; CARDOSO *et al*, 2015; WAGGONER *et al*, 2016b) dificultando a suspeita do diagnóstico clínico, principalmente em locais de co-circulação de arbovírus.

Além do quadro clínico similar entre as arboviroses dificultar o diagnóstico clínico, o conhecimento da ocorrência de um tipo de surto específico e a atenção que a mídia, a população e as autoridades sanitárias produz para a ocorrência deste surto, pode influenciar na suspeita clínica. Os nossos resultados corroboram com essa afirmação, apenas 10.7% dos pacientes com confirmação de infecção por CHIKV tiveram uma suspeita clínica correta registrada em prontuário médico. Um estudo descreve que durante o período da ocorrência do ZIKV em Salvador, já estava ocorrendo uma epidemia silenciosa de CHIKV, contudo as autoridades de saúde pública e os profissionais de saúde não detectaram imediatamente o aumento nos casos de CHIKV, provavelmente porque toda a atenção foi direcionada para o surto de ZIKV e complicações decorrentes (CARDOSO *et al*, 2017).

Como parte de conclusão dessa tese, chamamos a atenção para a frequência de dor articular persistente em pacientes, após infecção por chikungunya. A cada 10 indivíduos com infecção por CHIKV, 4 apresentaram dor articular por mais de 3 meses. Entretanto, esse estudo apresenta limitação que pode ter influenciado nesse achado: o período entre a inclusão do paciente no estudo na fase aguda da doença e o contato por telefone para investigação dos

sintomas crônicos, pode trazer um viés de memória, favorecendo uma subestimação ou superestimação da artralgia persistente.

Em Ilha da Reunião foi realizado um estudo longitudinal, após um surto de chikungunya e identificou que 60% dos indivíduos infectados por CHIKV podem ter manifestações crônicas por um período de 3 anos (SCHILTE *et al,* 2013). Outros autores, destacam que a fase crônica da doença pode ter uma duração de 5 anos (JELTE *et al,* 2017; RODRIGUEZ-MORALES *et al*, 2016). Em nosso estudo, 30.7% dos pacientes, permaneciam sintomáticos em média 1 ano e 5 meses. A diferença na frequência de persistência da dor articular após infecção por chikungunya, pode ser resultante das características específicas da cepa do CHIKV responsável pelos surtos, e das diferentes origens genéticas das populações estudadas (SCHILTE *et al,* 2013). Alternativamente, como a dor é considerada um sintoma subjetivo, pode refletir em uma diferença no relato da dor articular entre os pacientes.

Um estudo de coorte realizado em Curaçao, sugere que pacientes com infecção por dengue, concomitante com infecção por chikungunya, apresentam maior risco de desenvolver dores articulares persistente, se comparada a população infectada apenas por chikungunya (ELSINGA, 2018). Em nosso estudo ter dengue não foi um fator de risco associado a persistência da dor articular. Poucas investigações têm explorado essa hipótese, portanto, sugerimos que pesquisas sejam realizadas para esclarecer a fisiopatologia da infecção por chikungunya e dengue.

Os resultados desse estudo alertam para a capacidade da artralgia crônica e outras manifestações físicas e/ou psíquicas por um período prolongado, após a infecção por CHIKV, reduzir a capacidade dos indivíduos de desenvolver atividades diárias e laborais (COUTURIER *et al*, 2012; SOUMAHORO *et al,* 2008.). É importante destacar que pacientes com artralgia crônica precisam de acompanhamento de profissionais especializados, podendo trazer custos para o Sistema Único de Saúde (SUS). O clínico foi a especialidade mais procurada pelos pacientes com dor crônica em nosso estudo, mesmo sendo mais indicado atendimentos especializados, como serviços de reumatologia, fisioterapia e psicólogo. Tal situação pode ser explicada pela dificuldade de acesso à assistência especializada na rede pública e baixo poder aquisitivo da população estudada.

5 CONSIDERAÇÕES FINAIS

A co-circulação de DENV, CHIKV e ZIKV, a possibilidade de co-infecções, e um quadro clínico similar das infecções por arboviroses, apresenta uma série de desafios para o diagnóstico clínico preciso, condução médica adequada e notificação correta em áreas endêmicas. Isso demonstra a necessidade de obter dados sobre a epidemiologia, o quadro clínica das infecções por arboviroses, e um diagnóstico laboratorial preciso e acessível, para auxiliar na detecção correta da doença, cuidados clínicos adequados e colaborar com as ações de vigilância. Além disso, recomendamos que se estabeleça um sistema de vigilância sentinela para doenças febris e/ou outras doenças, em unidades de saúde, a fim de auxiliar na detecção precoce de novas epidemias.

Reforçamos a importância de se compreender e estudar a evolução da doença em pacientes confirmados por infecção por chikungunya, e os fatores associados ao risco de cronificação da artralgia, visto que observamos que a dor articular persistente influencia na qualidade de vida e na capacidade de desenvolver atividades diárias. Assim, são necessários estudos de seguimento, que busquem o acompanhamento dos pacientes com deficiências derivadas de sintomas reumatológicos, dentre outras manifestações físicas ou psíquicas, associadas a infecção por chikungunya. Por fim, sugerimos uma intervenção médica precoce e assistência de saúde adequada aos indivíduos, para reduzir a carga associada a doença.

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ANEXO

Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde
Instituto de Saúde Coletiva, Universidade Federal da Bahia
Termo de Consentimento Livre e Esclarecido
(para os participantes maiores de idade do grupo de referência no estudo)

Título do Projeto: Teste Rápido para Diagnóstico da Dengue: Avaliação Prospectiva do Desempenho e do Impacto no Manejo Clínico da Doença
Nome do Participante: |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__
No. de identificação do participante: |__|__|__|__|__|__|__|__|
Para ser lido por todos os participantes maiores de idade: As informações a seguir descrevem a pesquisa e o seu papel como participante. Por favor, leia com atenção e sinta-se à vontade para tirar qualquer dúvida com o entrevistador.
Objetivo da Pesquisa: Esta é uma pesquisa sobre a dengue. Esta pesquisa está sendo realizada pelo Centro de Pesquisas Gonçalo Moniz, da Fundação Oswaldo Cruz, Ministério da Saúde, e pelo Instituto de Saúde Coletiva, da Universidade Federal da Bahia. A dengue é um grande problema de saúde pública em Salvador e no Brasil. O diagnóstico da dengue na sua fase inicial é difícil, pois os sintomas como febre e dor no corpo são comuns a muitas doenças. Nós estamos convidando você a participar desta pesquisa porque você procurou este centro de saúde com sintomas que podem ser de dengue. Você pode ou não estar com dengue e para se ter certeza é necessário fazer um exame de sangue. Alguns dos exames de sangue usados para fazer o diagnóstico da dengue são complexos e podem demorar meses para ficarem prontos. Assim, o objetivo desta pesquisa é avaliar se um teste rápido, capaz de fazer o diagnóstico da dengue em 20 minutos, funciona tão bem quanto os outros exames mais demorados. Se você concordar, nós vamos fazer exames com o seu sangue, urina e saliva usando os testes para dengue usados habitualmente nos serviços de saúde, para comparar com os resultados de pessoas que também farão o teste rápido. O resultado de seus exames levará até três meses para ficarem prontos, mas assim que estiverem prontos eles serão encaminhados ao seu prontuário no posto de saúde para o benefício de seus cuidados médicos futuros e também poderão ser retirados por você aqui no posto. Adicionalmente, o seu sangue, urina e saliva poderão ser testados para outras doenças na tentativa de identificar a causa da doença que você apresentou. Você terá acesso aos resultados de todos os testes realizados assim que disponíveis. Talvez você não seja beneficiado diretamente pela sua participação nesta pesquisa, mas as informações que nós obtivermos poderão ajudar a melhorar os exames usados para o diagnóstico da dengue, permitindo que ele seja feito mais rápido no futuro.
Procedimentos a serem seguidos: Se você aceitar participar desta pesquisa, após ter entendido este termo de consentimento, o entrevistador fará perguntas a respeito de como você se sente e sobre a história da sua doença. Além disso, precisaremos coletar uma amostra de 10 mililitros (1 colher de sobremesa) do seu sangue. Você pode sentir um pouco de dor no local onde o sangue for coletado e mais raramente pode aparecer uma mancha roxa ou uma infecção no local, mas este risco será reduzido ao mínimo porque um profissional treinado irá realizar a coleta. O sangue coletado será utilizado para saber se você tem dengue. Você precisará retornar a este centro de saúde daqui a duas semanas para que possamos realizar uma nova entrevista e coletar uma segunda amostra de sangue em quantidade igual à primeira amostra de sangue. Caso você não retorne, agendaremos uma visita de nossa equipe à sua casa, para que a entrevista e a segunda coleta de sangue sejam realizadas em seu domicílio. Isso é importante porque nem sempre é possível confirmar o diagnóstico de dengue com apenas uma amostra de sangue e também porque poderemos coletar dados sobre a evolução de sua doença. Além disso, precisaremos coletar uma amostra da sua urina e saliva. A equipe de estudo também vai revisar o seu prontuário médico para obter informações sobre a conduta médica e a evolução de sua doença.

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Rubrica do pesquisador — Rubrica do participante do estudo

No. de identificação do participante: |__|__|__|__|__|__|__|__|

Confidencialidade: Suas respostas durante a entrevista e os resultados dos seus exames serão confidenciais. Apenas você, os responsáveis pelos seus cuidados médicos no posto de saúde, o grupo de pesquisadores deste estudo e os Comitês de Ética em Pesquisas terão acesso a estas informações. Você não será identificado em nenhum relatório ou publicação resultante da pesquisa. Qualquer informação que possa identificá-lo não será divulgada quando os resultados da pesquisa forem apresentados. Entretanto, os profissionais de saúde são obrigados a informar às secretarias de saúde sobre a identificação de casos de dengue. Por isso, se os seus exames forem positivos para dengue nós teremos que informar os resultados à secretaria de saúde.

Participação Voluntária: Sua participação nesta pesquisa é voluntária. Você pode se recusar a participar ou interromper sua participação em qualquer momento. Também, a equipe de estudo pode optar por encerrar sua participação durante ou no fim da pesquisa. Neste caso, você será avisado. Durante a entrevista, você tem todo o direito de se recusar a responder qualquer pergunta. Também pode se negar a fazer a coleta de sangue. A recusa em participar de todo ou de parte desta pesquisa não afetará seus cuidados médicos e nem haverá prejuízo em suas relações presentes ou futuras com o posto de saúde ou com as instituições envolvidas na pesquisa. Você não será responsável por nenhuma despesa associada com esta pesquisa e não receberá ajuda financeira para participar do estudo, mas você será ressarcido pela despesa relativa ao seu deslocamento ao centro de saúde no dia em que você retornar para coletarmos uma segunda amostra do seu sangue. Você receberá uma via deste termo de consentimento.

Grupo de Contato: Se no futuro você tiver qualquer dúvida sobre sua participação ou sobre seus direitos como participante na pesquisa, por favor, entre em contato com o Dr. Guilherme Ribeiro, Pesquisador do Centro de Pesquisas Gonçalo Moniz, Rua Waldemar Falcão, 121, Brotas, Salvador, telefone (71) 3176-2302, ou com o Comitê de Ética em Pesquisas, Centro de Pesquisas Gonçalo Moniz, Rua Waldemar Falcão 121, Brotas, Salvador, telefone (71) 3176-2285, e-mail: cep@bahia.fiocruz.br.

Consentimento: Eu entendi este termo de consentimento. Minhas perguntas foram devidamente respondidas. Sendo assim, eu voluntariamente concordo em participar do estudo.

Eu voluntariamente concordo que meu sangue, urina e saliva também sejam testados para as seguintes doenças:

Adenovírus:	☐ Sim ☐ Não	Citomegalovírus (CMV):	☐ Sim ☐ Não
Chikungunya:	☐ Sim ☐ Não	*Toxoplasma gondii:*	☐ Sim ☐ Não
Esptein Barr Vírus (EBV):	☐ Sim ☐ Não	*Mycoplasma pneumoniae:*	☐ Sim ☐ Não
Metapneumovírus humano (hMPV):	☐ Sim ☐ Não	*Haemophilus influenzae*:	☐ Sim ☐ Não
Parainfluenza (PIV) tipos 1 e 3:	☐ Sim ☐ Não	*Streptococcus pneumoniae*:	☐ Sim ☐ Não
Vírus influenza A e B:	☐ Sim ☐ Não	*Staphylococcus aureus*:	☐ Sim ☐ Não
Vírus sincicial respiratório (RSV):	☐ Sim ☐ Não	*Bordetella pertussis*:	☐ Sim ☐ Não
Zika vírus:	☐ Sim ☐ Não	Arboviroses:	☐ Sim ☐ Não

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Assinatura do participante do estudo Data Hora

☐ Impressão Digital do Participante do Estudo

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Assinatura do Investigador Data Hora

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Assinatura da Testemunha Data Hora

Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde
Instituto de Saúde Coletiva, Universidade Federal da Bahia
Termo de Assentimento

Título do Projeto: Teste Rápido para Diagnóstico da Dengue: Avaliação Prospectiva do Desempenho e do Impacto no Manejo Clínico da Doença
Nome do Participante: |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__
No. de identificação do participante: |__|__|__|__|__|__|__|
Para ser lido a todos os participantes menores de idade: As informações a seguir são referentes a uma pesquisa sobre a dengue que estamos realizando nesta unidade de saúde. Por favor, escute cuidadosamente e fique a vontade para fazer perguntas e tirar dúvidas com o entrevistador.
Nós estamos pedindo sua ajuda porque você tem uma doença que pode ser dengue. Nós gostaríamos de conversar com você sobre sua doença e fazer exames no seu sangue para que possamos descobrir se você está ou não com dengue. Estas informações poderão ajudar a melhorar o diagnóstico rápido da dengue no futuro.
Se você concordar em ajudar, nós faremos a você e a seus pais algumas perguntas sobre o que você está sentindo. Também gostaríamos de coletar uma pequena quantidade de sangue do seu braço com uma agulha e isso pode causar um pouco de dor no local onde o sangue for coletado e mais raramente pode aparecer uma mancha roxa ou uma infecção no local, porém este risco será muito pequeno porque um profissional treinado vai realizar a coleta. Daqui a duas semanas você terá que retornar ao posto de saúde ou então nós iremos visitar você em sua casa para fazer novas perguntas e mais uma coleta de sangue. Também gostaríamos de coletar uma pequena quantidade de sua saliva e urina. Seu sangue, urina e saliva serão examinados para que possamos dizer se você está com a dengue ou com outras doenças. Só faremos isso se você e seus pais estiverem de acordo. Você terá acesso aos resultados de todos os testes realizados assim que disponíveis.
Assentimento: Eu entendi este termo de assentimento. Minhas perguntas foram respondidas. Entendo que se eu não quiser participar, não sou obrigado, mesmo que os meus pais dêem seu consentimento. Eu, voluntariamente concordo em participar:
☐ Eu voluntariamente concordo que meu sangue, urina e saliva sejam testado para outras doenças além da dengue.

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Assinatura do Participante do Estudo	Data	Hora

☐ Impressão Digital do Participante do Estudo

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Assinatura do Investigador	Data	Hora

Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde
Instituto de Saúde Coletiva, Universidade Federal da Bahia
Termo de Consentimento Livre e Esclarecido
(para os responsáveis legais pelos participantes menores de idade do grupo de referência no estudo)

Título do Projeto: Teste Rápido para Diagnóstico da Dengue: Avaliação Prospectiva do Desempenho e do Impacto no Manejo Clínico da Doença

Nome do Participante: |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__

NO. DE IDENTIFICAÇÃO DO PARTICIPANTE: |__|__|__|__|__|__|__|

Para ser lido por todos os responsáveis legais pelos participantes menores de idade: As informações a seguir descrevem a pesquisa e o papel de seu filho (ou do indivíduo pelo qual você é o responsável legal) como participante. Por favor, leia com atenção e sinta-se à vontade para tirar qualquer dúvida com o entrevistador.

Objetivo da Pesquisa: Esta é uma pesquisa sobre a dengue. Esta pesquisa está sendo realizada pelo Centro de Pesquisas Gonçalo Moniz, da Fundação Oswaldo Cruz, Ministério da Saúde, e pelo Instituto de Saúde Coletiva, da Universidade Federal da Bahia. A dengue é um grande problema de saúde pública em Salvador e no Brasil. O diagnóstico da dengue na sua fase inicial é difícil, pois os sintomas como febre e dor no corpo são comuns a muitas doenças. Nós estamos convidando seu filho a participar desta pesquisa por ter procurado este centro de saúde com sintomas que podem ser de dengue. Ele pode ou não estar com dengue e para se ter certeza é necessário fazer um exame de sangue. Alguns dos exames de sangue usados para fazer o diagnóstico da dengue são complexos e podem demorar meses para ficarem prontos. Assim, o objetivo desta pesquisa é avaliar se um teste rápido, capaz de fazer o diagnóstico da dengue em 20 minutos, funciona tão bem quanto os outros exames mais demorados. Se você concordar, nós vamos fazer exames com o sangue, urina e saliva do seu filho usando os testes para dengue usados habitualmente nos serviços de saúde, para comparar com os resultados de pessoas de também farão o teste rápido. O resultado dos exames levará até três meses para ficarem prontos, mas assim que estiverem prontos eles serão encaminhados ao prontuário do seu filho no posto de saúde para o benefício de cuidados médicos futuros e também poderão ser retirados por você aqui no posto. Adicionalmente, o sangue, urina e saliva do seu filho poderão ser testado para outras doenças na tentativa de identificar a causa da doença que o seu filho apresentou. Você terá acesso aos resultados de todos os testes realizados assim que disponíveis. Talvez seu filho não seja beneficiado diretamente pela sua participação nesta pesquisa, mas as informações que nós obtivermos poderão ajudar a melhorar os exames usados para o diagnóstico da dengue, permitindo que ele seja feito mais rápido no futuro.

Procedimentos a serem seguidos: Se você aceitar que seu filho participe desta pesquisa, após ter entendido este termo de consentimento, o entrevistador fará perguntas a respeito de como ele se sente e sobre a história da doença. Além disso, precisaremos coletar uma amostra de 2 mililitros (1 colher de café) de sangue nos participantes com idade entre 6 meses e menores que 2 anos, 5 mililitros (1 colher de chá) de sangue nos participantes com idade entre 2 e 4 anos, e 10 mililitros (1 colher de sobremesa) de sangue nos participantes com idade maior ou igual a 5 anos. Seu filho pode sentir um pouco de dor no local onde o sangue for coletado e mais raramente pode aparecer uma mancha roxa ou uma infecção no local, mas este risco será reduzido ao mínimo porque um profissional treinado irá realizar a coleta. O sangue coletado será utilizado para saber se seu filho tem dengue. Você precisará trazer seu filho a este centro de saúde daqui a duas semanas para que possamos realizar uma nova entrevista e coletar uma segunda amostra de sangue em quantidade igual à primeira amostra de sangue. Caso você não retorne com o seu filho, agendaremos uma visita de nossa equipe à sua casa, para que a entrevista e a segunda coleta de sangue sejam realizadas no domicílio. Isso é importante porque nem sempre é possível confirmar o diagnóstico de dengue com apenas uma amostra de sangue e também porque poderemos coletar dados sobre a evolução da doença. Além disso, precisaremos coletar uma amostra de urina e saliva do seu filho. A equipe de estudo também vai revisar o prontuário médico do seu filho para obter informações sobre a conduta médica e a evolução da doença.

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Rubrica do pesquisador — Rubrica dos Pais ou Responsável Legal

No. de identificação do participante: |__|__|__|__|__|__|__|__|

Confidencialidade: Suas respostas durante a entrevista e os resultados dos exames serão confidenciais. Apenas você, os responsáveis pelos cuidados médicos do seu filho no posto de saúde, o grupo de pesquisadores deste estudo e os Comitês de Ética em Pesquisas terão acesso a estas informações. Seu filho não será identificado em nenhum relatório ou publicação resultante da pesquisa. Qualquer informação que possa identificá-lo não será divulgada quando os resultados da pesquisa forem apresentados. Entretanto, os profissionais de saúde são obrigados a informar às secretarias de saúde sobre a identificação de casos de dengue. Por isso, se os exames do seu filho forem positivos para dengue nós teremos que informar os resultados à secretaria de saúde.

Participação Voluntária: A participação do seu filho nesta pesquisa é voluntária. Você pode recusar que seu filho participe ou interromper a participação dele em qualquer momento. Também, a equipe de estudo pode optar por encerrar a participação durante ou no fim da pesquisa. Neste caso, você será avisado. Durante a entrevista, você e seu filho têm todo o direito de se recusar a responder qualquer pergunta. Também pode se negar a fazer a coleta de sangue. A recusa em participar de todo ou de parte desta pesquisa não afetará os cuidados médicos do seu filho e nem haverá prejuízo nas relações presentes ou futuras com o posto de saúde ou com as instituições envolvidas na pesquisa. Você não será responsável por nenhuma despesa associada com esta pesquisa e não receberá ajuda financeira pela participação do seu filho no estudo, mas você será ressarcido pela despesa relativa ao deslocamento ao centro de saúde no dia em que você retornar com o seu filho para coletarmos uma segunda amostra de sangue. Você receberá uma via deste termo de consentimento.

Grupo de Contato: Se no futuro você tiver qualquer dúvida sobre a participação do seu filho ou sobre os direitos dele como participante na pesquisa, por favor, entre em contato com o Dr. Guilherme Ribeiro, Pesquisador do Centro de Pesquisas Gonçalo Moniz, Rua Waldemar Falcão, 121, Brotas, Salvador, telefone (71) 3176-2302, ou com o Comitê de Ética em Pesquisas, Centro de Pesquisas Gonçalo Moniz, Rua Waldemar Falcão 121, Brotas, Salvador, telefone (71) 3176-2285, e-mail: cep@bahia.fiocruz.br.

Consentimento: Eu entendi este termo de consentimento. Minhas perguntas foram respondidas. Eu, voluntariamente, concordo que o paciente do qual sou responsável legal participe deste estudo.

Eu concordo que o sangue, urina e saliva do paciente do qual sou responsável legal também podem ser testados para as seguintes doenças:

Adenovírus:	☐ Sim	☐ Não	Citomegalovírus (CMV):	☐ Sim	☐ Não
Chikungunya:	☐ Sim	☐ Não	*Toxoplasma gondii:*	☐ Sim	☐ Não
Esptein Barr Vírus (EBV):	☐ Sim	☐ Não	*Mycoplasma pneumoniae:*	☐ Sim	☐ Não
Metapneumovírus humano (hMPV):	☐ Sim	☐ Não	*Haemophilus influenzae*:	☐ Sim	☐ Não
Parainfluenza (PIV) tipos 1 e 3:	☐ Sim	☐ Não	*Streptococcus pneumoniae*:	☐ Sim	☐ Não
Vírus influenza A e B:	☐ Sim	☐ Não	*Staphylococcus aureus*:	☐ Sim	☐ Não
Vírus sincicial respiratório (RSV):	☐ Sim	☐ Não	*Bordetella pertussis*:	☐ Sim	☐ Não
Zika vírus:	☐ Sim	☐ Não	Arboviroses:	☐ Sim	☐ Não

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Assinatura dos Pais ou Responsável Legal | Data | Hora

☐ Impressão Digital dos Pais ou Responsável

_____ _____ _____

Assinatura do Investigador | Data | Hora

_____ _____ _____

Assinatura da Testemunha | Data | Hora