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

Monaíse M. O Silva,[1] Laura B. Tauro,[1,2] Mariana Kikuti,[1,3] Rosângela O. Anjos[1], Viviane C. Santos,[1] Thaiza S. F. Gonçalves,[1] Igor A. D. Paploski,[3,4] Patrícia S. S. Moreira,[1] Leile C. J. Nascimento,[1] Gúbio S. Campos,[5] Albert I. Ko,[1,6] Scott C. Weaver,[7] Mitermayer G. Reis,[1,8] Uriel Kitron,[1,9] Guilherme S. Ribeiro[1,3,8]

[1]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil; [2]Instituto Nacional de Medicina Tropical, CONICET, Puerto Iguazú, Argentina; [3]Instituto de Saúde Coletiva, Universidade Federal da Bahia, Salvador, Brazil; [4]University of Minnesota, St. Paul, USA; [5]Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, Brazil; [6]Yale School of Public Health, New Haven, USA; and [7]University of Texas Medical Branch, Galveston, USA; [8]Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Brazil; [9]Emory University, Atlanta, USA.

Corresponding author: G. S. Ribeiro. Instituto Gonçalo Moniz, Fundação Oswaldo Cruz. Rua Waldemar Falcão, 121, Candeal, 40296-710 Salvador, BA, Brasil. (guilherme.ribeiro@bahia.fiocruz.br)

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 is more common with chikungunya. Nevertheless, correct medical diagnosis is challenging without laboratory testing.

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 northeastern 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%). An 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.

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

INTRODUCTION

Dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) viruses are widely distributed arboviruses, affecting tropical and sub-tropical areas [1]. In Brazil, the four DENV serotypes have co-circulated since 2010 [2], and in September 2014, the first autochthonous cases of chikungunya were reported in the country, almost simultaneously in the northern (Amapá state) and northeastern (Bahia state) regions [3,4]. 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 [5,6]. 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 [7].

Patients infected by DENV, CHIKV or ZIKV may develop an acute febrile illness, with similar clinical characteristics [8]. Symptoms and signs commonly observed include fever, rash, muscle pain, arthralgia, and headache [8]. Due to the difficulty in diagnosing arboviral infections based on clinical impressions, particularly in areas with concomitant co-circulation, laboratory tests are needed for accurate diagnosis of these viral agents. 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 [9]. 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 of co-circulation.

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

From September 2014 to July 2016, we conducted enhanced surveillance to detect DENV, CHIKV, and ZIKV infections among acute febrile patients attending a public emergency health unit in Salvador, as described in supplementary methods. 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 determine presumptive clinical diagnoses. Acute and convalescent blood samples were collected at study entry and 15 days later, respectively (detailed in supplementary methods). During the

follow-up for convalescent blood collection, a second interview was performed to obtain data on resolution of signs and symptoms.

Arboviral diagnosis

Acute sera, obtained as described in supplementary methods, underwent RNA extraction (Maxwell® Viral Total Nucleic Acid K), followed by reverse transcription-polymerase chain reaction (RT-PCR) for DENV [10], ZIKV [11], and CHIKV [12] with the AccessQuick™ RT-PCR System kit (Promega, USA). In addition, we performed DENV and CHIKV IgM-ELISA (Panbio Diagnostics, Brisbane, Australia and Inbios International Inc., Seattle, USA, respectively) on both acute and convalescent sera and tested the former with a DENV NS1-ELISA (Panbio Diagnostics). We did not employ a ZIKV serological assay due to the low accuracy of the available tests [13,14].

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 (Supplementary Table). 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 but 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.

RESULTS

Laboratory diagnosis of arboviral infection

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 infections, the expected frequency of DENV/CHIKV co-infection, assuming that specific arboviral infections were independent events, 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). The co-infection frequencies that we detected 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, 6 (0.6%) recent CHIKV among the 20 acute FLAV 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 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 based on signs and symptoms 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, between July and October 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 [3,4], though apparently major amplification in Salvador only began in June 2015, one month after the ZIKV epidemic peak in May 2015 [15]. Curiously, ZIKV spread in Salvador was very rapid and the outbreak, comprising ~17,500 case reports, lasted only two months [15,16], 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

[17]. Although our surveillance study included only one health unit of Salvador, our arboviral detection over time reflected previous citywide observations [15–17].

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 [18], inconsistent with the explosive amplification that was observed citywide [15]. 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, variation in the sequence and timing of human arboviral infections, and even the involvement of other *Aedes* species, such as *Ae. albopictus*, in ZIKV and CHIKV transmission.

We also found that co-infections were relatively common, affecting 23 (9.3% of the 247 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. The impressively high frequencies of concomitant and sequential infections were apparently due to the intense simultaneous transmission of the three arboviruses in Salvador during the study period.

Statistically, the likelihood of simultaneously detecting two independent events is estimated by multiplying their individual likelihood detection. However, two events could also be dependent, e.g. the co-infection of people by two different arboviruses through the bite of a

mosquito carrying more than one arbovirus, resulting in simultaneous transmission. In our study, the observed frequencies of human co-infections were not statistically different from those expected, under the assumption of independent arboviral infections. This negative finding suggests that human co-infections are non-associated, rather than dependent events. However, as our non-association findings are supported merely by statistical analyses, they might not represent the true behavior of these viruses in nature. Further studies are needed to investigate potential arbovirus interactions in vectors and hosts, and to better determine whether pathogenesis and clinical outcomes of 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 limited capacity for detecting ZIKV infections among the general patient population seeking medical care at the health unit because our inclusion criteria required the presence of fever, which is less common in ZIKV infections [19]. In addition, the sensitivity of ZIKV molecular diagnosis is limited [20], hampering case detection during the viremic phase of the infection, and we did not employ ZIKV serological tests due to their low accuracy [13,14]. 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. It is important to emphasize that FLAV infections were most likely caused either by DENV or ZIKV, as there are no reports of other FLAV causing human infections in Salvador. Yet, we cannot completely rule out the possibility of silent circulation of other FLAV pathogens, such as yellow fever or West Nile, causing unrecognized infections.

Interestingly, Zika patients had a lower median age compared other arboviral-infected patients. As ZIKV infections typically 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 older adults are less prominent than in children and younger adults, as previously observed in a Puerto Rico study that showed that, among individuals with laboratory-confirmed ZIKV infections, those who were symptomatic were younger than those who were asymptomatic [21]. Previous DENV exposures, which increase with age, may play an immunolomodulatory role in this difference [22,23].

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 [24] and Nicaragua [8], while arthralgia were more common in chikungunya patients, as reported in Trinidad [25]. 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 [17]. 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 during 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.

Acknowledgments

We thank the technical staff who participated in data collection and sample processing; Renan Rosa and Perla Santana for their assistance with data management and administrative matters; the São Marcos Health Unit staff for providing health assistance to the patients; and the patients for their participation in the study.

Funding

This work was supported by the Brazilian National Council for Scientific and Technological Development [grants 400830/2013-2, and 440891/2016-7 to G.S.R.; and scholarships to I.A.D.P., L.B.T., U.K., M.G.R., and G.S.R.]; the Bahia Foundation for Research Support [PET0026/2013, APP0044/2016, and PET0022/2016 to G.S.R., and scholarship to M.M.O.S.]; the Coordination for the Improvement of Higher Education Personnel, Brazilian Ministry of Education [440891/2016-7 to G.S.R. and scholarship to M.K.]; the National Institutes of Health (USA) [R01 TW009504, R01 AI121207, and U01 AI088752 to A.I.K.], [R24 AI AI120942 to SCW]; the Yale School of Public Health; the Federal University of Bahia; and the Oswaldo Cruz Foundation.

Conflict of interest: SCW owns intellectual property related to chikungunya vaccine development and has two patents for alphavirus vaccine development issued. AIK has a patent on methods and composition for detection of flavivirus infection pending.

References:

1. Wilder-Smith A, Gubler DJ, Weaver SC, Monath TP, Heymann DL, Scott TW. Epidemic arboviral diseases: priorities for research and public health. Lancet Infect Dis. **2017**;17:e101–6.

2. Temporão JG, Penna GO, Carmo EH, et al. Dengue virus serotype 4, Roraima State, Brazil. Emerg Infect Dis. **2011**;17:938–40.

3. Nunes MRT, Faria NR, de Vasconcelos J, et al. Emergence and potential for spread of Chikungunya virus in Brazil. BMC Med. **2015**;13:1–10.

4. Teixeira MJ, Andrade AMS, Costa MC, et al. East/Central/South African Genotype Chikungunya Virus, Brazil, **2014**. Emerg Infect Dis J. 2015;21:906–7.

5. Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. Emerg Infect Dis. **2015**;21:1885–6.

6. Zanluca C, de Melo VCA, Mosimann ALP, dos Santos GIV, dos Santos CND, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz. **2015**;110:569–72.

7. Aliota MT, Bassit L, Bradrick S S, et al. Zika in the Americas , year 2 : What have we learned ? What gaps remain ? A report from the Global Virus Network. Antiviral Res. **2017**;144:223–46.

8. Waggoner JJ, Gresh L, Vargas MG, et al. Viremia and Clinical Presentation in Nicaraguan Patients Infected With Zika Virus , Chikungunya Virus , and Dengue Virus. Clin Infect Dis. **2016**;63:1584–90.

9. Nkengasong JN, Nsubuga P, Nwanyanwu O, et al. Laboratory systems and services are

critical in global health: Time to end the neglect? Am J Clin Pathol. **2010**;134:368–73.

10. Lanciotti R, Calisher C, Gubler D, Chang G, Vorndam A. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol. **1992**;30:545–51.

11. Balm MND, Lee CK, Lee HK, Chiu L, Koay ESC, Tang JW . A diagnostic polymerase chain reaction assay for Zika virus. J Med Virol. **2012**;84:1501–05.

12. Edwards CJ, Welch SR, Chamberlain J, et al. Molecular diagnosis and analysis of Chikungunya virus. J Clin Virol. **2007**;39:271–5.

13. L'Huillier AG, Hamid-Allie A, Kristjanson E, et al. Evaluation of Euroimmun Anti-Zika Virus IgM and IgG Enzyme-Linked Immunosorbent Assays for Zika Virus Serologic Testing. J Clin Microbiol. **2017**;55:2462–71.

14. Kikuti M, Tauro LB, Moreira PSS, et al. Diagnostic performance of commercial IgM and IgG enzyme-linked immunoassays (ELISAs) for diagnosis of Zika virus infection. Virol J. **2018**;15:1–7.

15. Cardoso CW, Paploski IAD, Kikuti M, et al . Outbreak of Exanthematous Illness Associated with Zika, Chikungunya, and Dengue Viruses, Salvador, Brazil. Emerg Infect Dis J. **2015**;21:2274–6.

16. Paploski IAD, Prates APPB, Cardoso CW, et al. Time Lags between Exanthematous Illness Attributed to Zika Virus, Guillain-Barré Syndrome, and Microcephaly, Salvador, Brazil. Emerg Infect Dis J. **2016**;22:1438–44.

17. Cardoso CW, Kikuti M, Prates APPB, et al. Unrecognized Emergence of Chikungunya Virus during a Zika Virus Outbreak in Salvador , Brazil. PLoS Negl

Trop Dis. **2017**;11:e0005334.

18. Roundy CM, Azar SR, Rossi SL, et al. Variation in aedes aegypti mosquito competence for zika virus transmission. Emerg Infect Dis. **2017**;23:625–32.

19. Duffy MR, Chen T-H, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. **2009**;360:2536–43.

20. Fischer C, Pedroso C, Mendrone A, et al. External Quality Assessment for Zika Virus Molecular Diagnostic Testing, Brazil. Emerg Infect Dis. **2018**;24:1–5.

21. Lozier MJ, Burke RM, Lopez J, et al. Differences in Prevalence of Symptomatic Zika Virus Infection , by Age and Sex — Puerto Rico , 2016. J Infect Dis. **2018**;217:1678-1689.

22. Wen J, Elong Ngono A, Angel Regla-Nava, et al. Dengue virus-reactive CD8+T cells mediate cross-protection against subsequent Zika virus challenge. Nat Commun. **2017**;8:1–11.

23. Priyamvada L, Quicke KM, Hudson WH, et al. Human antibody responses after dengue virus infection are highly cross-reactive to Zika virus. Proc Natl Acad Sci. **2016**;113:7852–7.

24. Azeredo EL, dos Santos FB, Barbosa LS, et al. Clinical and Laboratory Profile of Zika and Dengue Infected Patients : Lessons Learned From the Co- circulation of Dengue , Zika and Chikungunya in Brazil. PLOS Curr Outbreaks. **2018**;2:1–27.

25. Sahadeo N, Mohammed H, Allicock OM, et al. Molecular Characterisation of Chikungunya Virus Infections in Trinidad and Comparison of Clinical and Laboratory Features with Dengue and Other Acute Febrile Cases. PLoS Negl Trop Dis. **2015**;9:1–

18.

Characteristics	DENV (N: 32)	CHIKV (N: 159)	ZIKV (N: 13)	FLAV (N: 20)	DENV/CHIKV (N: 13)	CHIKV/FLAV (N: 9)	Negative (N: 568)

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.

	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 titles.

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.

Footnote. Of the 247 cases of acute arboviral infection, 39 showed evidence of a recent arboviral infection. These are in addition to the other 133 recent arboviral infections shown in the figure.

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

Figure 1

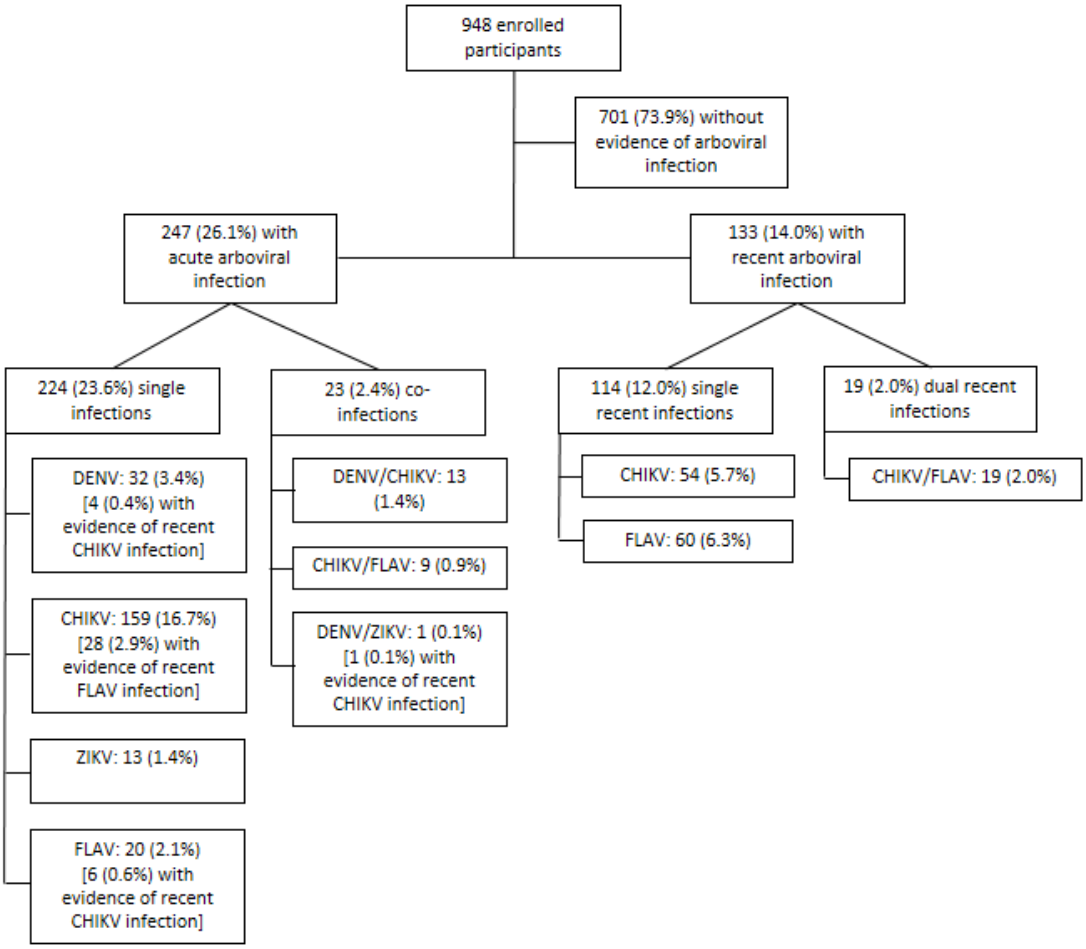

Figure 2