Journal of Clinical Virology 113 (2019) 27–30

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Undetected Chikungunya virus co-infections in a Brazilian region presenting hyper-endemic circulation of Dengue and Zika

Flávia Fonseca Bagno[a,b], Maria Marta Figueiredo[b], Jannely Villarreal[c], Glauco de Carvalho Pereira[d], Lara Carvalho Godoi[b,e], Flávio Guimarães da Fonseca[a,b,*]

[a] Departmento de Microbiologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[b] Centro de Tecnologia de Vacinas, Belo Horizonte, Brazil
[c] Department of Molecular and Cellular Biology, Harvard University, Cambridge, United States
[d] Fundação Ezequiel Dias (FUNED), Belo Horizonte, Brazil
[e] Colégio Técnico da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

ARTICLE INFO

Keywords:
Chikungunya
Arboviruses
Diagnosis
Dengue
Zika
Co-infection

ABSTRACT

Background: Chikungunya virus (CHIKV) causes a disease characterized by acute onset of fever accompanied by arthralgia. Clinical similarities and co-circulation of other arboviruses such as Dengue virus (DENV) and Zika virus (ZIKV), have complicated their differentiation, making their diagnoses a challenge for the health authorities. Misdiagnosis is a serious issue to the management of patients and development of public health measures.
Objectives: We carried out further screening of CHIKV, DENV and ZIKV cases in Minas Gerais, Brazil, after diagnostics were already issued by a state laboratory and according to the Brazilian Ministry of Health (BMH) policy. Our aim was to look for possible co-infections or previous arboviruses' exposure.
Study design: Sera from 193 patients with symptoms of arboviral infections were tested for DEV, ZKV and/or CHIKV by the State laboratory, according to clinical suspicion and following standard BMH guidelines. After an official diagnosis was issued for each patient, we retested samples applying a broader panel of ELISA-based serological tests.
Results: We identified 13 patients with concurrent or consecutive infections (IgM positive for more than one arbovirus), including 11 individuals that were positive for CHIKV and other previously confirmed arbovirus infection.
Discussion: Guidelines established in many arbovirus-endemic countries prioritizes the diagnosis of Zika and Dengue and no further analyzes are done when samples are positive for those viruses. As a result, possible cases of co-infections with chikungunya are neglected, which affects the epidemiological assessments of virus circulation, patient management, and the development of public health policies.

1. Background, objectives and study design

Chikungunya virus (CHIKV), an arbovirus of the Alphavirus genus within the *Togaviridae* family, causes a disease characterized by the acute onset of fever, hash and long-lasting arthralgia. CHIKV has also been associated with cases of encephalopathy (primarily in neonates) and Guillain–Barré Syndrome (GBS) [1]. Similarly to *Dengue virus* (DENV) and *Zika virus* (ZIKV) - arboviruses from the *Flaviviridae* family - CHIKV is transmitted to humans mainly by *Aedes* mosquitoes. Clinical similarities and co-circulation of these arboviruses have complicated their differentiation, making their diagnoses a challenge for the health authorities in regions where they co-circulate. To complicate matters, there is an extensive serological cross-reaction between DENV and ZIKV [2–4], and some cross-reaction between flaviviruses and CHIKV have also been described [5]. The incorrect or incomplete diagnosis in such scenario affects the development of prevention and treatment strategies [6]. In Brazil, for instance, during outbreak season the Brazilian Ministry of Health (BMH) prioritizes the diagnostic of Dengue due to its

Abbreviations: CHIKV, Chikungunya virus; DENV, Dengue virus; ZIKV, Zika virus; ELISA, enzyme-linked immunoassays; BMH, Brazilian Ministry of Health; GBS, Guillain-Barré Syndrome; MG, Minas Gerais; LACEN, Laboratory of Public Health (*Laboratório Central de Saúde Pública*); FUNED, Ezequiel Dias Foundation (*Fundação Ezequiel Dias*)

* Corresponding author at: Universidade Federal de Minas Gerais (UFMG), Av. Pres. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG, Brazil.
E-mail addresses: flavia.bagno@gmail.com (F.F. Bagno), mariamartafigueiredo@gmail.com (M.M. Figueiredo), jvillarreal@college.harvard.edu (J. Villarreal), glaiconep@gmail.com (G.d.C. Pereira), lcarvalhogodoi@gmail.com (L.C. Godoi), fdafonseca@icb.ufmg.br (F.G. da Fonseca).

https://doi.org/10.1016/j.jcv.2019.02.006
Received 20 December 2018; Received in revised form 25 February 2019; Accepted 26 February 2019

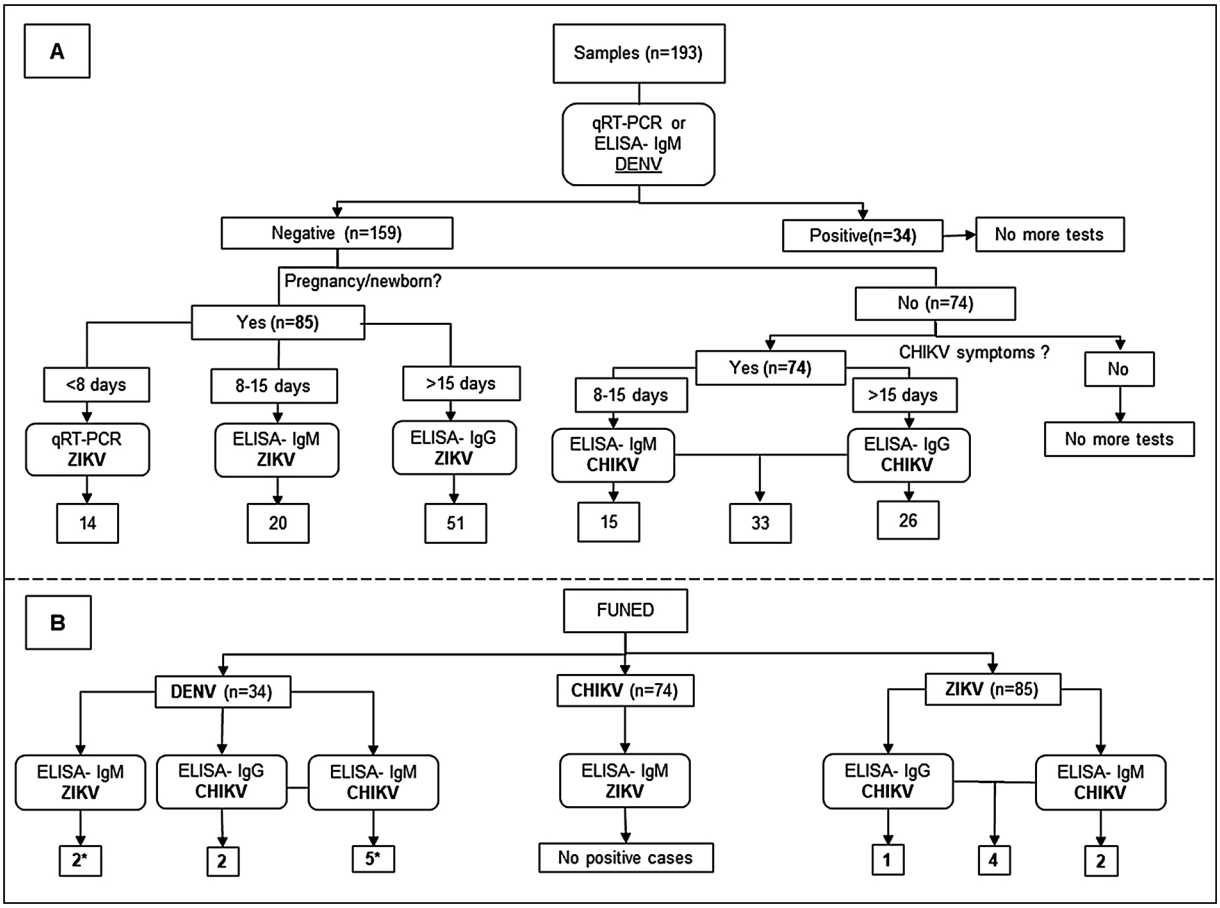

Fig. 1. Cases of previously undetected Chikungunya co-infections in a panel of 193 sera from patients tested by a Federal Laboratory in Brazil. A: Tests performed by LACEN-FUNED according to Brazilian Ministry of Health guidelines, which recommend to perform tests against DENV for all suspected cases of arboviral diseases. ZIKV diagnostic are considered a priority in cases of suspected infections in pregnant women. Possible CHIKV are investigated in cases of DENV-negative samples or when patients present high fever, onset of unexplained arthralgia or acute arthritis. When tested positive for a particular arbovirus, samples are not re-tested for other viruses. B: Complementary tests, showing the number of positive results for two or more viruses. We identified 11 patients presenting possible concurrent infections (positive IgM) by DENV/CHIKV (n = 5) or ZIKV/CHIKV (n = 6) (showed in black boxes). Two cases of DENV-ZIKV co-infections were detected, including one patient presenting positive serology for all three infections. * Contains the triple-positive patient (CHIKV/DENV/ ZIKV - IgM).

severity [7]. In recent years, the Zika diagnostic has also been made a priority in case of suspected infections in pregnant women and in their babies.

We investigated the presence of antibodies against CHIKV, DENV, and ZIKV in sera of patients with confirmed arboviral infections. These sera were previously tested by the Central Laboratory of Public Health (*Laboratório Central de Saúde Pública* - LACEN) at Ezequiel Dias Foundation (*Fundação Ezequiel Dias* -FUNED), Minas Gerais (MG), Brazil, which follows directives from the BMH (Fig. 1A). Our study provided a complementary characterization of these samples in order to detect non-identified cases of co-infections or previous infections by a different arbovirus (Fig. 1B).

One-hundred and ninety-three serum specimens, received in October of 2017 to early 2018 from several cities of MG, Brazil, were analyzed at FUNED for epidemiological assessments. These were investigated according to the described clinical symptoms of each patient and epidemiological criteria. All samples were initially tested for Dengue. Tests for Zika were only performed in samples from pregnant women, newborns with microcephaly, or patients with neurological symptoms. According to BMH guidelines, CHIKV tests were only performed in DENV-negative samples or in suspected cases of Chikungunya Fever, which must include the onset of unexplained arthralgia or acute arthritis [7,8]. Once tested positive for a particular arbovirus, samples are not retested for other viruses. We reevaluated all sera using in house and commercial enzyme-linked immunoassays (ELISA) (Euroimmun, Germany) to look for possible co-infections or previous arbovirus exposure. Differently from the BHM guidelines, every sample was re-screened for CHIKV, DENV and ZKV independently of clinical and epidemiological criteria.

2. Results

Following the BMH guidelines, 74 patients out of 193 tested positive for CHIKV, 34 were positive for DENV, and 85 for ZIKV (Fig. 1A). In our complementary screening (Fig. 1B) we detected 11 concurrent infections (IgM-positive), by DENV/CHIKV (n = 5) or ZIKV/CHIKV (n = 6). Importantly, only one amongst these 11 potential CHIKV coinfection cases was previously picked-up by the public laboratory. Additionally, two DENV-infected patients presented IgG for CHIKV, and this may simply indicate previous exposure to the later virus. Considering the 10 newly identified CHIKV samples, they were initially classified only as ZIKV-positives (7,1% of the ZIKV cases) or as DENV-positives (14,7% of the DENV cases). We also detected one case of DENV-ZIKV concurrent /consecutive infection, and one patient presented positive serology for all three infections (IgM-positive for DENV, CHIKV and ZIKV).

Samples with suspect of Dengue or Chikungunya infections are sent to LACEN along with a specific on-line clinical notification formulary (*Sistema de Informação de Agravos de Notificação* - SINAN). The main symptoms described for the seropositive CHIKV patients were fever (81%), arthralgia (73%), and myalgia (65%) (Fig. 2). On the other

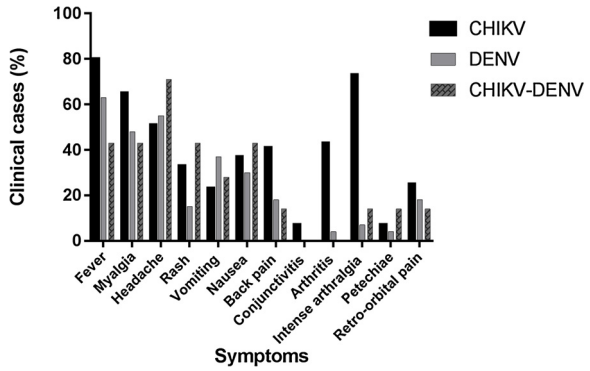

Fig. 2. Incidence of symptoms of patients with Chikungunya, Dengue and both infections. The main symptoms of positive CHIKV patients were fever, intense arthralgia, and myalgia. On the other hand, patients with concurrent CHIKV and DENV infection, presented, mostly, headache. Different for mono-infected CHIKV patients, which presented high incidence of arthralgia, this was not a significative symptom present in CHIK-DENV patients.

hand, main clinical presentations in the newly identified CHIKV-DENV co-infected/consecutively infected patients seemed to be slightly different: 81% of these patients presented headache and 43% of them presented fever, myalgia, rash and nausea. Different from mono-infected CHIKV patients, which presented high incidence of arthralgia, only one of the CHIK-DENV patients presented this symptom (73% and 14%, respectively). With the exception of one patient that tested positive only for CHIKV-IgM (but positive for IgG in the complementary screening), these positive CHIKV-DENV patients were not previously tested against CHIKV by LACEN. This kind of analysis was not conducted for the ZKV-positive patients because the forms for these cases are less detailed than those from Dengue and Chikungunya, and included only pregnant woman and their respective newborns.

Considering the total number of ZIKV-CHIKV co-detection, four patients (57%) are from the same city - Governador Valadares (GV) - that had confirmed cases of all three infections in the past years. The city is in the northeastern part of MG and is an important interpost connecting the southern Brazil to the Northeast part of the Country, which was a hotspot for the massive ZIKV outbreak the year before (Fig. 3). One of the ZIKV/CHIKV-positive patients was a one-year-old baby presenting symptoms of GBS (the patient was CHIKV-IgM and CHIKV-IgG positive). A second ZIKV-positive baby, a newborn with microcephaly, was also CHIKV-IgG positive. It was not possible to correlate these results with serology from their respective mothers, and we cannot rule out the possibility that such antibodies (especially the anti-CHIKV IgGs) may have come from them.

3. Discussion

We investigated the presence of antibodies against CHIKV, DENV, and ZIKV in the sera of patients with suspected arboviral infection, previously tested by LACEN at FUNED, which follows directives from the BMH. Our study provided a complementary characterization of these samples in order to detect non-identified cases of co-infections or previous infections by different arboviruses. We found 13 patients with possible concurrent/consecutive infections (IgM positive for more than one arbovirus), including 11 that were positive for CHIKV and for other previously detected arbovirus infection. As mentioned above, Minas Gerais state and particularly its northern region are geographically centered between the Northern and Southern Brazil, and the flow of infectious diseases within the Country can be effectively sampled in the State. The first autochthonous case of Chikungunya fever in MG was confirmed in 2016 [9]. In 2017, the state registered the highest number of suspect cases of the disease in the Country: 16,013 [10]. In that period, 10 deaths caused by the CHIKV were registered, including 10 from GV, the city where we found a high incidence of co-infections (see Fig. 3).

Earlier studies have reported co-infections of CHIKV/ZIKV [11–14] and CHIKV/DENV [14–17]. A common mosquito vector and socio-geographic settings contribute to the viruses 'co-circulation and co-infections in hyper-endemic regions [16]. Nonetheless, the BMH guidelines prioritizing the diagnosis of DENV for the general population and ZIKV in cases of pregnant women suspectedly infected may have created a bias, leading to the under notification of CHIKV cases. This happens because when a patient is serologically confirmed to be DENV-

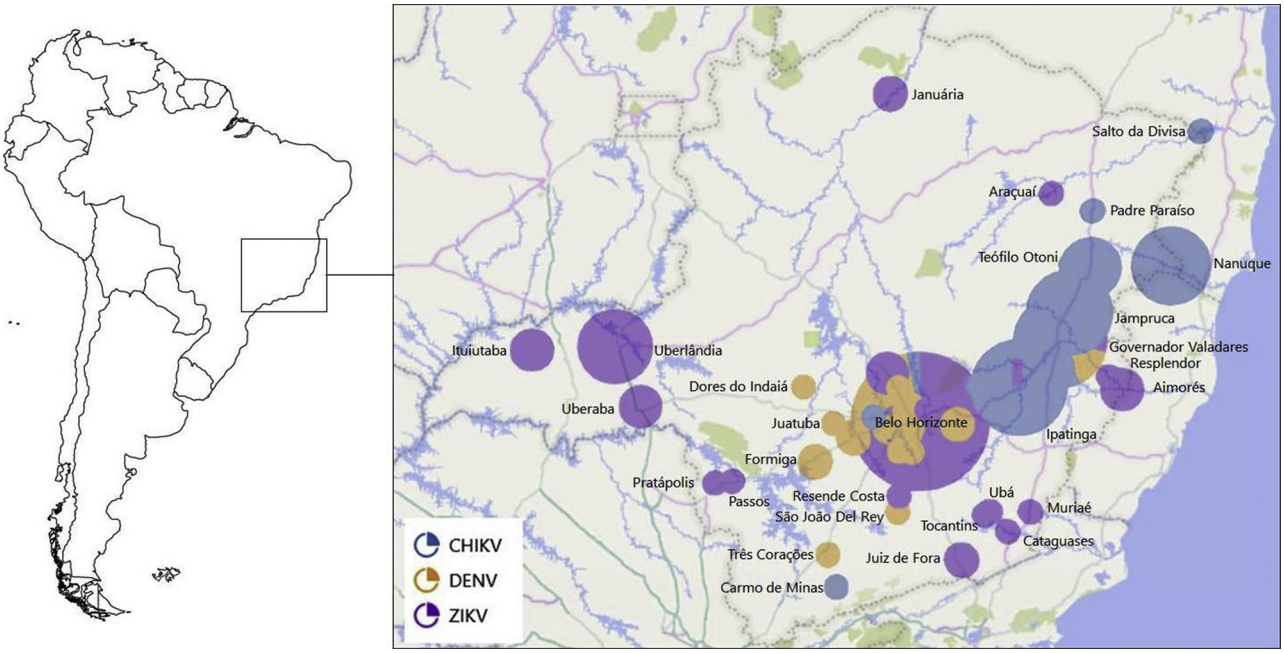

Fig. 3. (Legend): Origen of the samples tested in this study. Distribution of cases of Chikungunya (CHIKV, blue), Dengue (DENV, orange) and Zika (ZIKV, purple), by city of registration considering the period of investigation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

or ZIKV-positive, they are usually not further screened for possible co-infections. Indeed, as mentioned before, only one out of 11 newly identified CHIKV potential co-infections was detected by the reference Laboratory as it followed the BMH policy.

Two main aspects should be taken under consideration in relation to the possible CHIKV under notification in the case of co-infections with either DENV or ZIKV. First, would CHIKV co-infection impact the patient's clinical disease, especially when severe manifestations, such as GBS or other neurological complications are considered? Secondly, would the CHIKV under notification impact epidemiological assessments of the virus circulation and consequently affect policy-making and patient management in hyper-endemic regions? We believe the answer is "yes" in both cases.

Author Contribution

Bagno contributed to conceptualizing and designing the study, performed the assays, data analyses and drafted the manuscript. Figueiredo, Godoi and Villarreal performed the assays and revised the manuscript. Pereira provided the patient samples from the Central Laboratory of Public Health (LACEN) and contributed to revising the manuscript. Da Fonseca contributed to the conceptualization and design of the study, revised the manuscript, and supervised the study as the corresponding author.

Conflicts of interest

The authors have no conflict of interest to declare.

Ethical Approval

Ethical approval from Institutional Boards were not required for this study because all further serological testing were conducted in complementation to the official diagnostic proceedings carried out at the Central Laboratory of Public Health (LACEN-FUNED) in Belo Horizonte, Minas Gerais, Brazil.

Acknowledgements

We thank Marcos Vinícius F. Silva and Talita Émile R. Adelino (*Fundação Ezequiel Dias*, Brazil) for helping with the sera samples. We thank Edel F Barbosa-Stancioli and the Centro de Tecnologia em Vacinas' team (CTVacinas, Brazil) for discussions. This work was supported by FAPEMIG - grants # CBB-APQ-03081-17 (EDITAL 001/2017) and # CBB- APQ-04295-17 (EDITAL 007/2017).

References

[1] R. Mehta, P. Gerardin, C.A.A. de Brito, C.N. Soares, M.L.B. Ferreira, T. Solomon, The neurological complications of chikungunya virus: a systematic review, Rev. Med. Virol. 28 (3) (2018) e1978, https://doi.org/10.1002/rmv.1978.

[2] L. Priyamvada, W. Hudson, R. Ahmed, J. Wrammert, Humoral cross-reactivity between Zika and dengue viruses: implications for protection and pathology, Emerg. Microbes Infect. 6 (2017) e33, , https://doi.org/10.1038/emi.2017.42.

[3] S. Watanabe, N.W.W. Tan, K.W.K. Chan, S.G. Vasudevan, Dengue and zika virus serological cross-reactivity and their impact on pathogenesis in mice, J. Infect. Dis. (2018), https://doi.org/10.1093/infdis/jiy482.

[4] Neto, N.Medicine, F. Maia, J. Zacarkim, M.R. Queiroz, I.T. Labeaud, A. D. & Aronoff, D. 2018. Cross-Reactivity Between Zika and Dengue Virus: A Cross-Sectional Analysis in Rio Grande do Norte, Brazil. Open Forum Infectious Diseases. Neto, N., Medicine, F., Maia, J., Zacarkim, M. R., Queiroz, I. T., Labeaud, A. D., & Aronoff, D. 5(Suppl 1), S167. https://doi.org/10.1093/ofid/ofy210.454.

[5] S.K. Mardekian, A.L. Roberts, Diagnostic options and challenges for dengue and chikungunya viruses, BioMed Res. Int. (2015), https://doi.org/10.1155/2015/834371.

[6] M.R. Donalisio, A.R.R. Freitas, A.P.B. Von Zuben, Arboviruses emerging in Brazil: challenges for clinic and implications for public health, Rev. Saude Publica. 51 (30) (2017), https://doi.org/10.1590/S1518-8787.2017051006889.

[7] T.S. Salles, T.E. Sá-Guimarães, E.S.L. De Alvarenga, V. Guimarães-Ribeiro, M.D.F. De Meneses, P.F. De Castro-Salles, M.F. Moreira, History. Epidemiology and diagnostics of dengue in the American and Brazilian contexts: a review, Parasit. Vectors 11 (2018) 1–12, https://doi.org/10.1186/s13071-018-2830-8.

[8] Brazil Ministry of Health. Secretary of Health Surveillance. Department of Surveillance of Communicable Diseases. Chikungunya: Clinical Management (in Portuguese), (2017), pp. 5–47 (Accessed 25 January 2019), http://bvsms.saude.gov.br/bvs/publicacoes/chikungunya_manejo_clinico.pdf.

[9] Brazil. Ministry of Health of Brazil. Secretariat of Health Surveillance. Department of Noncommunicable Diseases Surveillance and Health Promotion. Health Brazil 2015/2016: an Analysis of Health Situation and the Epidemic Caused by Zika Virus and Other Diseases Transmitted by Aedes aegypti, (2017) (Accessed 25 January 2019), http://bvsms.saude.gov.br/bvs/publicacoes/health_brazil_2015_2016.pdf.

[10] Brazil Ministry of Health. Secretariat of Health Surveillance. Dengue, chikungunya and Zika virus epidemiological bulletin, (2018) EW 43 of 2018. (in Portuguese).http://portalarquivos2.saude.gov.br/images/pdf/2018/novembro/13/boletim-epidemiologico.pdf (Accessed 25 January 2019)..

[11] F.F. Norman, S. Chamorro, A. Vazquez, M.P. Sanchez-Seco, J.-A. Perez-Molina, B. Monge-Maillo, et al., Sequential Chikungunya and Zika virus infections in a traveler from Honduras, Am. J. Trop. Med. Hyg. 95 (2016) 1166–1168, https://doi.org/10.4269/ajtmh.16-0426.

[12] S. Sardi, S. Somasekar, S.N. Naccache, A.C. Bandeira, L.B. Tauro, G.S. Campos, et al., Co-Infections from Zika and Chikungunya virus in Bahia, Brazil identified by metagenomic next-generation sequencing, J. Clin. Microbiol. 54 (2016) 2348–2353, https://doi.org/10.1128/JCM.00877-16.

[13] H. Zambrano, J.J. Waggoner, C. Almeida, L. Rivera, J.Q. Benjamin, B.A. Pinsky, Zika virus and Chikungunya virus co-infections: a series of three cases from a single center in Ecuador, Am. J. Trop. Med. Hyg. 95 (2016) 894–896, https://doi.org/10.4269/ajtmh.16-0323.

[14] M.Y. Carrillo-Hernández, J. Ruiz-Saenz, L.J. Villamizar, S.Y. Gómez-Rangel, M. Martínez-Gutierrez, Co-circulation and simultaneous co-infection of dengue, chikungunya, and zika viruses in patients with febrile syndrome at the Colombian-Venezuelan border, BMC Infect. Dis. 18 (61) (2018), https://doi.org/10.1186/s12879-018-2976-1.

[15] T. Edwards, L.D. Signor, C. Williams, E. Donis, L.E. Cuevas, et al., Co-infections with Chikungunya and dengue viruses, Guatemala,2015, Emerg. Infect. Dis. 22 (2016) (2016) 2003–2005, https://doi.org/10.3201/eid2211.161017.

[16] M. Hisamuddin, A. Tazeen, M. Abdullah, M. Islamuddin, N. Parveen, Co-circulation of Chikungunya and Dengue viruses in Dengue endemic region of New Delhi, India during 2016, Epidemiol. Infect. (2018) 1–12, https://doi.org/10.1017/S0950268818001590.

[17] D. Taraphdar, A. Sarkar, B.B. Mukhopadhyay, S. Chatterjee, Short report: A comparative study of clinical features between monotypic and dual infection cases with chikungunya virus and dengue virus in West Bengal, India. Am. J. Trop. Med. Hyg. 86 (4) (2018) 720–723, https://doi.org/10.4269/ajtmh.2012.11-0704.