
# Co-circulating Arboviruses in Latin America:

# Zika Virus, Chikungunya Virus and Dengue Virus

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## DECLARATION OF WORK

I, Ludmila Lobkowicz, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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## LIST OF PUBLICATION IN THESIS

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**ABSTRACT**

Chikungunya (CHIKV), Dengue (DENV) and Zika (ZIKV) viruses have been of growing public health concern in Latin America. Increasing incidence of new infections alongside the continuing lack of licenced antivirals or vaccines have contributed to a rising burden of disease in populations and cost for healthcare systems. These burdens are further exacerbated due to the difficulty of achieving accurate diagnosis in settings where these viruses co-circulate. Thus, the aim of this research was to study co-circulating CHIKV, DENV and ZIKV in Latin America, particularly in relation to co-infections and the accurate identification of specific arbovirus infections.

First, a systematic review of the published literature on ZIKV co-infections was conducted, assessing the co-infection frequency among ZIKV infected cases and the impact of co-infection on the clinical presentation of ZIKV. Second, the co-circulation of CHIKV and ZIKV in a cohort of pregnant women in Recife, Brazil from 2015-2017 was described and the potential to differentiate between infections at symptom presentation was assessed.

The systematic review's main findings showed that the most frequent ZIKV co-infections occurred with CHIKV and DENV, and in some circumstances occurred in up to half of the ZIKV infections. Additionally, co-infection did not seem to affect the mild clinical presentation of ZIKV infections. However, the review was not able to assess a potential increase of complications associated with ZIKV co-infections compared to ZIKV mono-infections. Furthermore, the analysis of the cohort study showed that CHIKV and ZIKV infection were distinguishable upon clinical presentation in pregnant women.

Our findings on ZIKV co-infections and the clinical presentation of ZIKV and CHIKV infected pregnant women contribute to improved patient management in settings of arbovirus co-circulation, through aiming to facilitate clinical diagnosis and guide laboratory testing, in order to administer appropriate follow up if needed, and consequently to reduce complications associated with arbovirus infection.

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### 1. CHAPTER 1: LITERATURE REVIEW



### 2. CHAPTER II: METHODS & RESULTS

#### 2.1 Paper I



#### 2.2 Paper II

**ACRONYMS**

| | |
|---|---|
| ADEM | Acute disseminated encephalomyelitis |
| AFI | Acute febrile illness |
| ANC | Antenatal care |
| CDC | Center for Disease Control and Prevention |
| CHIKV | Chikungunya virus |
| Cievs/ PE | Center for Strategic Information on Health Surveillance in Pernambuco |
| CMV | Cytomegalovirus |
| DENV | Dengue virus |
| DHF | Dengue haemorrhagic fever |
| DSS | Dengue shock syndrome |
| EM | Epidemiological month |
| EW | Epidemiological week |
| GBS | Guillain-Barré syndrome |
| HIV | Human immunodeficiency virus |
| HSV | Herpes simplex virus |
| Ig | Immunoglobin |
| IUGR | Intrauterine growth restriction |
| LAVITE-FIOCRUZ | Laboratorio de Virologia e Terapia Experimental of the Fundação Oswaldo Cruz |
| MERG | Microcephaly Epidemic Research Group |
| SINAN | Sistema de Informação de Agravos de Notificação (i.e. Notifiable disease Information system) |
| TORCH | Toxoplasmosis, Others agents (e.g., Syphilis), Rubella, CMV, HSV |
| WNV | West Nile virus |
| YFV | Yellow fever virus |
| ZIKV | Zika virus |

# 1. CHAPTER 1: LITERATURE REVIEW

In this chapter, I provided a brief outline of the literature on arthropod-borne viruses (arboviruses). The key areas that are summarized are the epidemiology, transmission, clinical presentation, diagnosis, treatment, co-infection and infection in pregnancy of arboviruses, in particular of Chikungunya virus (CHIKV), Dengue virus (DENV) and Zika virus (ZIKV). Finally, the knowledge gaps which have driven this research are highlighted.

Arboviruses are a growing global public health concern[1]. This is provoked by the rising contribution of arbovirus infections to global disability and mortality over the past 50 years[2,3]. Furthermore, over the past 20 years arboviruses have been increasingly occurring either in an endemic manner or in explosive emergent and re-emergent epidemics in Latin America[4,5]. CHIKV, DENV and ZIKV are the arboviruses of greatest recent public health concern in Latin America[6]. Their rising public health relevance is due to their increasing prevalence and ongoing co-circulation over the past 20 years, and to the continuing lack of optimal tools for prevention (e.g., vaccines) and treatment (e.g., antivirals) of infections. The three arboviruses share the same mosquito vectors of the *Aedes* species (e.g., *Aedes aegypti, Aedes albopictus*), leading them to occur in highly overlapping geographic areas, predominately in urban settings. Furthermore, prevention of CHIKV, DENV and ZIKV infections is challenging as mosquito bites cannot be entirely avoided. Avoiding mosquito bites is especially challenging in low socio-economic status households due to lack of household protective measures, including unscreened houses and the absence of air-conditioning[7,8]. CHIKV, DENV and ZIKV have also been described as sharing similar clinical symptoms, which therefore makes differential diagnosis, supportive care and prognosis difficult, negatively impacting the health outcomes of patients and pregnancies. Arbovirus infections are generally asymptomatic and mild, typically presenting with fever and rash[1,9]. However, various neurological complications have been reported to result from arboviral infections[10].

## 1.1 Arboviruses

The defining feature of all arboviruses is their transmission between an arthropod vector and a vertebrate host[11]. Some arboviruses circulate in sylvatic cycles, characterized by their survival in wild

animals such as non-human primates (e.g., monkeys), birds, horses and rodents after having been transmitted by an arthropod vector, such as a mosquito or tick. To note, although CHIKV, ZIKV and DENV can circulate in sylvatic cycles, they are not dependent on them[12]. Additionally, arboviruses are primarily RNA viruses[11]. The high mutation rate of RNA viruses may be advantageous when these viruses alternate cycles of replication between very diverse environments such as invertebrate arthropods and vertebrates[11]. Arboviruses are taxonomically diverse, mainly originating from the families of *Flaviviridae, Togaviridae* and *Bunyaviridae*[10]. The geographic distribution of DENV and ZIKV from the family of *Flaviviridae* and CHIKV from the family of *Togaviridae* almost entirely overlap (Figure 1)[10], as the study by Charlier and colleagues did not display ZIKV cases in Asian countries between 2010-2019, such as those reported in Bangladesh, Cambodia, China, India, Malaysia, Indonesia, Japan, Maldives, Lao, Korea, Pakistan, Singapore, Myanmar, Philippines, Taiwan, Thailand and Vietnam[13].

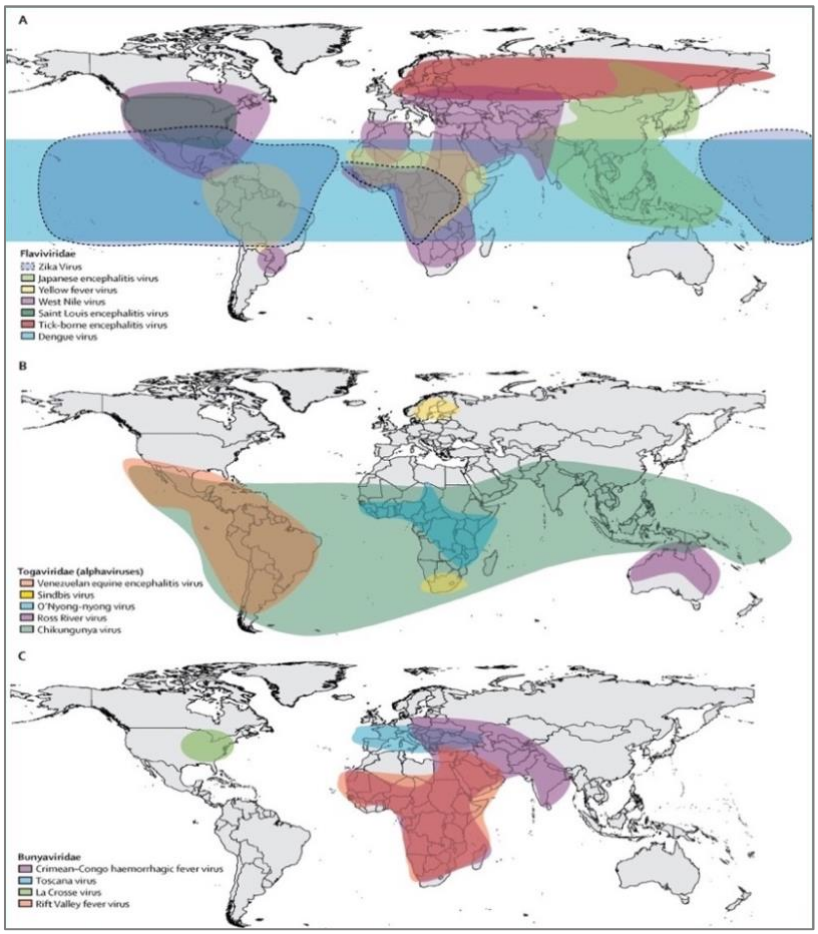


Figure 1: **World distribution of arboviruses.** Taken from Charlier *et al.* (2017)[10]. To highlight, in **A** the distribution of Zika virus is marked in light blue surrounded with a dashed line, and the distribution of Dengue virus in turquoise. In **B** Chikungunya virus is marked in green.

The increasing global prevalence of arboviruses is caused by a combination of factors. These factors include:

- Growing urbanisation and deforestation, which enables increased vector-host contact[11].
- Expanded human movement, which means that mosquito eggs and infected humans will spread to other previously unaffected areas that will in turn allow the vector to spread and will present previously uninfected mosquitos to become infected vectors[14].
- Poor sanitation conditions, such as no access to running water, which results in the population storing water, that consequently serves as additional mosquito breeding-sites, complicates vector control[14].
- Rising insecticide resistance of mosquitoes additionally challenges measures to control vector populations[14].
- Changing climate and climatic events, such as El-Niño, assist vector amplification and expansion beyond tropical latitudes[11].

The changing global climate and human demography also enhances the potential of new arboviruses emerging from sylvatic cycles to cause disease in animals and humans.

In the last 20 years, CHIKV, DENV and ZIKV have been the arboviruses of increasing public health concern in Latin America[6]. Their growing prevalence is causing a rising burden across the whole population[15]. In order to explain how CHIKV, DENV and ZIKV managed to spread almost globally, I describe their epidemiology below.

### 1.2 Epidemiology of CHIKV

CHIKV is an *Alphavirus* from the family of *Togaviridae,* which was first identified in Tanzania in 1953[16]. The name "Chikungunya" originates from a word used by the southeast Tanzanian Makonde ethnic group, which directly translated means "that which bends up", describing the patient's position when suffering from severe joint pains[17]. CHIKV, has four different genotypes: Asian, West African, East/Central/South African, and Indian Ocean[18]. The virus has a long history of emergence in urban transmission cycles, enzootic (i.e., circulating in an animal population) and sylvatic foci in sub-Saharan Africa[11,19]. From 2005, there have been several CHIKV outbreaks in the Indian Ocean Islands, South

East Asia, and Europe, where it is transmitted by *Aedes albopictus*. The most recent CHIKV outbreak started in Latin America in 2013[20-29]. In 2016, there was evidence of CHIKV transmission in 94 countries worldwide[30]. To date, about 1.3 billion people are estimated to be at risk of CHIKV infection[30,31]. This estimate is based on the population living in areas most environmentally suitable for mosquitoes, which are competent of CHIKV transmission[30,31].

### 1.3 Epidemiology of DENV

DENV is a *Flavivirus*, and consists of four different serotypes (DENV-1,-2,-3,-4). Historical reports describe dengue-like outbreaks in Latin America 400 years ago[32]. The name "Dengue" is thought to have originated from the Swahili term "ki-denga pepo" translating to "a disease characterized by the sudden cramp-like seizures caused by an evil spirit"[33]. DENV was first isolated in Japan in 1943 and then in Hawaii in 1945[34]. Various outbreaks took place simultaneously, and cases were reported that presented with dengue-like symptoms from India to the Pacific islands[35]. World War II is documented to be the origin of the global expansion of DENV[36]. Thousands of DENV cases within the Japanese and allied forces, in addition to the movement of their troops and war materials, enabled the virus and main vector *Aedes aegypti* to spread to most areas of Asia and the Pacific, where it had not been prevalent before[36]. In Latin America, an *Aedes aegypti* eradication program effectively eliminated this mosquito type in 23 countries during the 1950s and 1960s[37,38]. Although, the eradication program was initially aimed at the epidemic of the Yellow fever virus (YFV) it also effectively controlled the ongoing DENV epidemics[37]. However, the termination of this program in the 1970s led to the reestablishment of *Aedes aegypti in* the tropical areas of Latin America[36]. The program's termination along with increased urbanisation and the new introduction of DENV-3 in 1963, DENV-1 in 1977, DENV-4 from Asia in 1981, resulted in all four serotypes becoming endemic in Latin America[36]. In 2012, evidence revealed that 3.97 billion people in 128 countries were living with the risk of DENV infection[39]. This estimate, similarly to that of the population at risk of CHIKV infections, is based on the population living in areas most environmentally suitable for mosquitoes, which are competent of DENV transmission. However, in comparison to the risk of CHIKV infections measured at a 5km x5km spatial scale, the estimate of the risk of DENV infections was derived on a national level[30,31,39].

The actual number of DENV cases is believed to be underreported and many cases are misclassified due to similar clinical presentation of other febrile disease-causing pathogens[23]. A report in 2013 estimated that there were 390 million DENV infections per year worldwide (95% credible interval 284–528 million), of which 96 million (95% credible interval 67–136 million) manifested any symptomatic disease[40].

### 1.4 Epidemiology of ZIKV

During a YFV surveillance study, ZIKV was first isolated from the serum of a sentinel rhesus macaque in 1947 in the Ziika forest in Uganda, from which the virus's name originates. Subsequently, the *Flavivirus* ZIKV was isolated in Uganda from an *Aedes africanus* mosquito in 1948 and from humans in 1952[41-44]. In the following 60 years very few cases of ZIKV were diagnosed in Africa and Asia, leading to the assumption that ZIKV infection was mostly asymptomatic or caused mild febrile illness or was in very low transmission[44-49]. The first ZIKV disease outbreak was documented in 2007 on Yap Islands in the South Pacific, where approximately 73% of the population were infected (i.e., more than 900 infected inhabitants)[50]. After increasing cases throughout the Pacific Islands, the second largest ZIKV outbreak followed in French Polynesia in 2013-2014. Here, for the first time retrospective reports were presented of neurological complications in adults, such as Guillain-Barré syndrome (GBS)[51]. There is one ZIKV serotype (i.e., classification based on viral cell surface antigen) with two ZIKV lineages (African and Asian) and three ZIKV genotypes (i.e., a classification based on the viral genetic constitution) (West African, East African, and Asian)[45,52,53]. Both outbreaks, on Yap Island and in French Polynesia were caused by the Asian ZIKV lineage[54,55]. Although phylogenetic studies indicate virus introduction as early as 2013, the first confirmed case of ZIKV infection in the Americas, also caused by the Asian lineage, was reported in Northeast Brazil in May 2015[56,57]. ZIKV rapidly spread across Brazil, causing up to 1.5 million cases by early 2016[58]. The outbreak continued until late 2017, spreading to more than 87 other countries and territories worldwide [59-61]

## 1.5 Transmission of CHIKV, DENV and ZIKV

There are several different routes of transmission of the arboviruses CHIKV, DENV and ZIKV (Table 1). The first transmission route is by mosquito bite[1]. CHIKV, DENV and ZIKV share the same vector, the *Aedes spp.* mosquitoes[1]. Notably, all arboviruses that experienced the most striking emergence in the 21st century in Latin America (i.e., ZIKV, DENV and CHIKV) are transmitted in urban or peri-urban (i.e., areas immediately surrounding cities) areas by the *Aedes spp.* mosquitoes, and mainly by *Aedes aegypti*[1]. *Aedes aegypti* is now predicted to be present in almost all tropical and subtropical areas (Figure 2), and over 3 billion people are currently living in regions where *Aedes* is present[62].

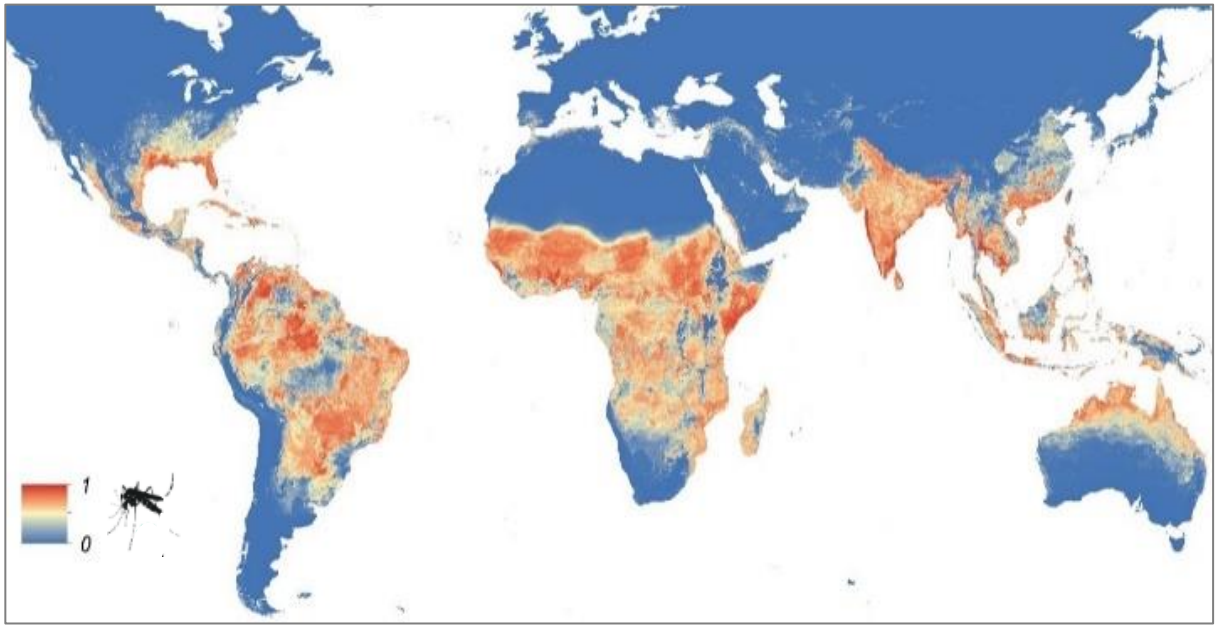


Figure 2: **Global map of the predicted distribution of *Aedes aegypti*.** Map depicts probability of occurrence (from 0 blue to 1 red). Adapted from Kraemer *et al.* (2015)[62].

After an incubation period of the arbovirus within the mosquito *Aedes aegypti*, which typically ranges from 3 to 14 days, a female mosquito will develop a persistent salivary gland infection and generally remains infectious for a lifetime[63]. The main source of virus for uninfected mosquitoes is infected symptomatic and asymptomatic humans, as they are the main carriers and multipliers of the virus. All three viruses have also been reported to rarely be transmitted by blood transfusion, and ZIKV is the only *Flavivirus* to date that has been confirmed to be sexually transmitted[36,64,65]. Further, mother-to-child transmission has been reported for all three arboviruses, but the transmission frequency and mechanisms seem to differ between CHIKV, DENV and ZIKV. CHIKV has been

described to be primarily transmitted in the periods (i.e., -7 days to -3 days prior-to-delivery) and the intrapartum periods (i.e., -2 days prior-to-delivery to +2 days post-delivery), although congenital (i.e., >7 days prior-to-delivery) transmission has also been described[66-69]. Congenital and antepartum transmission of DENV have been reported with similar frequnency[70-74]. In contrast, ZIKV seems to be mainly transmitted congenitally, specifically via transplacental transmission, although some antepartum transmission has been documented[65].

## 1.6 Clinical presentation of CHIKV, DENV and ZIKV

The clinical presentations of CHIKV, DENV and ZIKV have been described to be very similar. However, frequencies of symptoms of the respective viruses are currently unspecified. Studies estimating the proportion of asymptomatic cases of CHIKV, DENV and ZIKV infections show a wide range of results. Hence, between 60% and 75% of DENV and ZIKV infections and 3% to 75% of CHIKV infections have been estimated to be asymptomatic[75-78]. Overall, there is significant overlap in the clinical symptoms of the three arboviruses, their incubation period, symptomatic period as well as the duration of the period when viral RNA persists in serum (Table 1)[79]. However, CHIKV and ZIKV infections have been described to not be characterised by bleeding, and DENV infections have only rarely been described to present with conjunctivitis[79,80]. Further, all three arboviruses are associated in rare cases with complications of infection, such as encephalopathy, encephalitis, myelitis, acute disseminated encephalomyelitis (ADEM) and GBS. A specific complication of CHIKV is a chronic stage characterized by unpredictable relapses, which include sensation of fever, muscular weakness, and worsening of joint stiffness as well as general viral polyarthropathy, which is defined as pain and inflammation in four or more joints. Most DENV complications occur due to a mechanism called antibody-dependent enhancement (ADE), which is enhanced disease severity due to a secondary infection caused by a different DENV serotype to the primary infection[81]. One specific DENV complication is Dengue haemorrhagic fever (DHF), which is characterized by plasma leakage of different severity levels. DHF can lead to Dengue shock syndrome (DSS), causing severe plasma leakage that can lead to shock in the patient. About 10% of all DENV cases have been reported to develop DHF or DSS[82]. ZIKV has an unusual tropism (i.e., specificity of virus for a particular host cell)

*Table 1:* ***Transmission and clinical presentation of CHIKV, DENV and ZIKV***

| | **CHIKV** | **DENV** | **ZIKV** |
|---|---|---|---|
| **Transmission** | - Mosquito bite[a,83]<br>- Blood transfusion<br>- Mother-to-child[84] | - Mosquito bite[a,83]<br>- Blood transfusion<br>- Mother-to-child[85] | - Mosquito bite[a]<br>- Blood transfusion<br>- Mother-to-child[86]<br>- Sexually |
| **Estimated symptomatic cases among infected** | 25-97%[76,78] | 25%[75] | 38.2%<br>(95% CI: 13.9-67.0%)[77] |
| Incubation period[b]<br>Duration of symptoms[b] | 3-12 days,<br>7-10 days, | 4-10 days<br>2-7 days, | 3-14 days,<br>2-7 days |
| Median period of viral RNA in serum | 1-6 days[76] | 1-6 days[79] | 1-6days[79] |
| **Clinical symptoms** | | | |
| Rash | Yes | Yes | Yes |
| Fever | Yes | Yes | Yes |
| Arthralgia | Yes | Yes | Yes |
| Myalgia | Yes | Yes | Yes |
| Headache | Yes | Yes | Yes |
| Retro-orbital pain | Yes | Yes | Yes |
| Conjunctivitis | Yes | Yes | Yes |
| Lymphadenopathy | Yes | Yes | Yes |
| Oedema in limbs | Yes | Yes | Yes |
| Bleeding | No | Yes | No |
| **Complications** | Encephalopathy &Encephalitis, Myelitis, Guillain-Barré syndrome,<br>Acute disseminated encephalomyelitis,<br><br>Myelopathy, Neuroocular disease, Encephalomyelo-neuropathy,<br><br>Viral polyarthropathy, Polyarthralgia, Polyarthritis, Tenosynovitis, Raynaud syndrome,<br><br>Adverse birth outcomes[c] | Encephalopathy & Encephalitis, Myelitis, Guillain-Barré syndrome,<br>Acute disseminated encephalomyelitis,<br><br>Meningitis, Stroke, Cerebellar syndrome,<br><br>Dengue haemorrhagic fever, Dengue shock syndrome,<br><br>Adverse birth outcomes[c] | Encephalopathy & Encephalitis, Myelitis, Guillain-Barré syndrome,<br>Acute disseminated encephalomyelitis,<br><br>Meningoencephalitis, Seizures, Sensory polyneuropathy,<br><br>Adverse birth outcomes[c] |

*[a]by Aedes agypti or Aedes albopictus, [b]Incubation period= time of exposure (or infection) to symptom onset, Duration of symptoms= time of ongoing symptoms. [c]See table 2.*

for progenitor neural cells in the developing human foetus, resulting in clusters of neurodevelopmental birth defects in approximately 5-10% of ZIKV infections in pregnancy[86-93]. Further, this neurotropism also causes a number of severe neurological complications in children and adults, which seem to be caused by both direct neuro-invasion (e.g., encephalitis) and post-infectious autoimmunity (e.g., GBS)[1].

### 1.7 Diagnostics

Diagnostic testing of CHIKV, DENV and ZIKV infections can be accomplished using both molecular and serological methods, but, as explained below, the choice of method depends on the number of days from infection or symptom onset (Figure 3)[94]. Thus, asymptomatic infections can make diagnostics challenging.

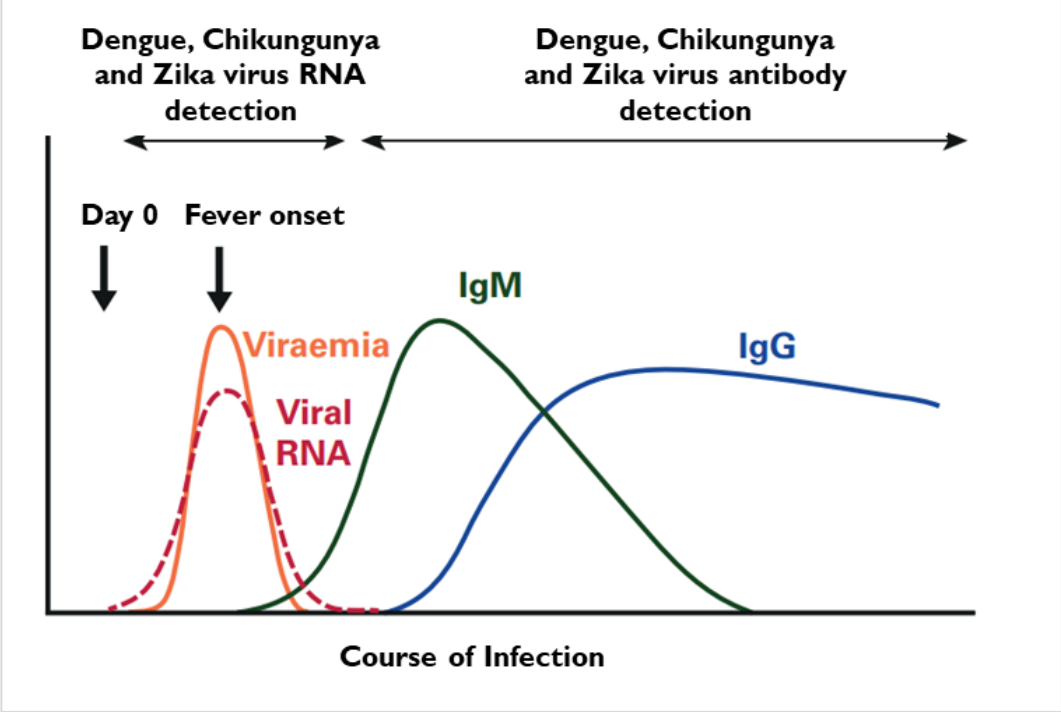


Figure 3: **Diagnostics of Chikungunya virus, Dengue virus and Zika virus.** *Time of molecular and serological testing during course of a primary infections of* Chikungunya virus, Dengue virus and Zika virus. *Adapted from EUROIMMUN*[94].

While viral RNA of CHIKV, DENV and ZIKV has been reported to persist for longer in some patients, viral clearance in the bloodstream during a typical infection occurs around 7 days after symptom onset (Table 1)[76,79,95,96]. Thus, molecular testing for viral RNA (e.g., quantitative real time

polymerase chain reaction (qRT-PCR)) is predominantly conducted within the first 7 days of symptom onset (Figure 3)[97]. CHIKV, DENV and ZIKV RNA have also been detected in urine samples[98,99]. In fact, the WHO even suggests PCR testing in urine samples for ZIKV RNA for up to 30 days[97]. Specifically for ZIKV, RNA can also be detected in semen for up to 60 days post symptom onset[97].

Samples collected from patients after 7 days of symptom onset are subjected to molecular and primarily serological diagnostic testing[97]. Serological testing can include testing titres of Immunoglobin (Ig) M antibodies (i.e., from about 5 days to 12 weeks after symptom onset), IgG antibodies (i.e., from about 10 days to 6 months for ZIKV and CHIKV and for several years for DENV), which are both tested by antibody enzyme-linked immunosorbent assays (ELISA), or testing neutralizing antibodies using a plaque reduction neutralization test (PRNTs) (Figure 3) [97,100-102] [103-105]. However, there is a principal obstacle to ELISA serological testing: inherent serological cross-reactivity exhibited by the *Flavivirus* species, due to high frequency of common antibody epitopes[106]. Thus, depending on the validation cohort (e.g., cases from areas of high flavivirus co-circulation vs. travellers), IgM and IgG ZIKV ELISAs have been reported to indicate wide ranges of specificity (i.e., true negative rate) and sensitivity (i.e., true positive rate)[102]. In addition, in patients with previous DENV infection, the initial antibody response upon ZIKV infection has been described to be a DENV IgG response instead of a ZIKV IgM response, thus further reducing diagnostic sensitivity[107]. To validate ELISA results, the "gold standard" diagnostic for flaviviruses can be performed, which is the PRNTs[103-105]. Although this technique requires elaborate training and specialised facilities and is very labour-intensive and expensive, it is currently the only diagnostic tool to accurately differentiate viral infections. The evaluation of seroconversion is an additional diagnostic method of arbovirus infection. This is conducted by taking two consecutive samples and testing them by either IgM or PRNT[108]. Seroconversion by IgM can be confirmed, if there is a switch from negative status in the first sample to positive status in the second sample[108]. Seroconversion by PRNT can be confirmed by a rise in PRNT titers between the two samples or a switch from negative status in the first sample to positive status in the second sample[108].

## 1.8 Treatment and vaccines

To date, there is no licenced antiviral therapeutic for CHIKV, DENV or ZIKV infections. Treatment of symptoms is the only clinical resource to manage CHIKV, DENV and ZIKV infected patients. These treatments include rest, hydration and specific pain medication. DENV infected patients should only receive acetaminophen (i.e., paracetamol), and should strictly avoid aspirin and ibuprofen, as these nonsteroidal anti-inflammatory drugs (NSAIDs) can cause a mild DENV clinical presentation to develop into a severe DENV clinical presentation, which may require hospitalisation and sometimes even intensive care treatment[109]. After a DENV infection has been ruled out, CHIKV and ZIKV patients can be treated with NSAIDs in addition to acetaminophen[110-112].

In contrast to DENV, there are no approved CHIKV and ZIKV vaccines to date, although a number of CHIKV and ZIKV vaccines are currently under trial[113-118]. For DENV, a live attenuated vaccine, chimeric yellow fever 17D-tetravalent dengue vaccine (CYD-TDV) has been licensed by the U.S. Food and Drug Administration[119]. However, in 2017 the vaccine manufacturer, Sanofi Pasteur, announced that people who receive the CYD-TDV vaccine without previously having been DENV infected may be at risk of developing severe DENV fever if they become DENV infected after being vaccinated[120]. These adverse vaccine outcomes led to the vaccine being exclusively administered to an age group of 9 to 45 years with documented confirmed previous DENV infection. Nevertheless, those most at need of DENV vaccines are the paediatric cases (i.e., 1 to 15 years of age) in endemic DENV regions, as DENV fever and DHF mainly affect children under 15 years of age[121]. Thus, the licensed vaccine is of limited use. As such, the seven DENV vaccine candidates currently in trial, including an additional two live attenuated vaccines, an inactivated virus vaccine, a recombinant subunit vaccine, a viral vectored vaccine, and two DNA vaccines are of great importance[122].

## 1.9 Co-infection of arboviruses

The circulation of arboviruses in tropical and subtropical areas, where the prevalence of other infectious pathogens is high, leads to an increased risk of co-infection with co-circulating arboviruses and other infectious diseases[123]. Co-infected patients can present with similar clinical manifestations to monotypic infected (mono-infected) patients, which complicates diagnosis[124]. Misdiagnosis or

missed diagnosis of one or more of the multiple infecting agents restricts epidemiological understanding of co-infection, which has serious potential implications for the health outcomes of infected patients. For example, misdiagnosing a DENV as a CHIKV infection or missing a DENV co-infection may lead to inappropriate prescription of arthralgia alleviating NSAIDs. These are usually used for CHIKV patients, but, as previously described, lead to severe bleeding in DENV patients with thrombocytopenia or DHF[125]. A systematic review on CHIKV/DENV co-infections describes the clinical presentation of co-infections in four studies. However these studies were of limited methodological quality[126]. Three of those studies, a case report and two cross-sectional studies, found neither symptoms nor clinical outcomes of co-infections (n=85 cases) were exacerbated in relation to mono-infections[126-129]. The fourth, a hospital-based case series by Chahar and colleagues found a high rate of severe symptoms and poor clinical outcomes among co-infected patients (n=6 cases), but no details were provided regarding the clinical presentation of DENV or CHIKV mono-infected patients, to allow comparison[130].

Moreover, the extent to which co-infection could enhance disease severity remains unclear. Vogels and colleagues recently hypothesized various scenarios of how co-infections could act on arboviral replication and associated pathology[131].

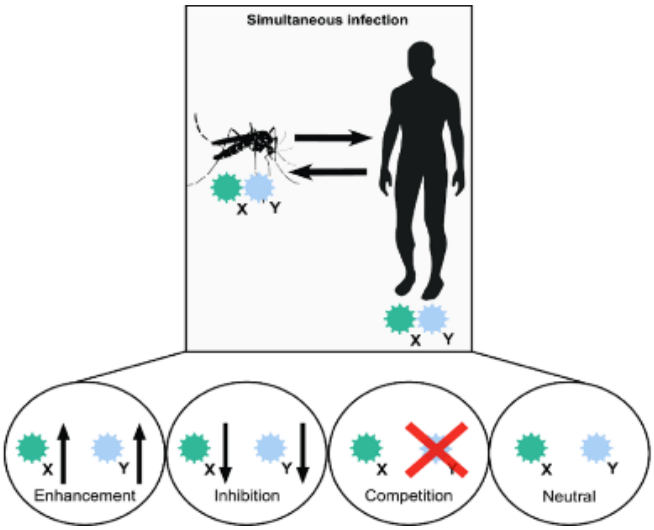


Figure 4: **Possible scenarios of impact of co-infection on arbovirus replication and associated pathology.** Extracted from Vogels *et al.* (2019)[131].

These scenarios are depicted in Figure 4 and include:

- **Enhancement** of viral replication and following pathology.
- **Inhibition** of viral replication and following pathology.
- **Competition** between the virus and the co-infecting agent, resulting in viral replication and pathology identical to mono-infection of the "winning" agent.
- **Neutral** relationship between virus and co-infecting agent, no effect on viral replication or pathology.

Enhancement of pathology could either occur through an increased viral replication due to simultaneous interaction with the immune system by multiple pathogens, or an exacerbated immune response to an increased viremia[131]. Vogels and colleagues describe possible enhanced virus replication through a CHIKV/DENV co-infection inhibiting two fundamental antiviral responses simultaneously (e.g., CHIKV interferes with the nuclear transport of signal transducer and activator of transcription 1 (STAT1) and DENV blocks STAT2 phosphorylation[132]. STAT1 and STAT2 are two transcription factors involved in interferon signalling). Additionally, a cellular exonuclease that degrades viral RNA, 5′-3′ exoribonuclease 1 (XRN1), may promote replication of flaviviruses, such as ZIKV and DENV, when co-infecting the same cell[133]. Finally, endothelial permeability during DENV infection may change tissue tropism of co-infecting viruses to enhance viral pathology[134].

An alternative potential consequence of co-infection could be the triggering of an increased immune response, which would lead to reduced overall viremia and consequently to reduced disease severity, resulting in overall inhibition of pathology[131]. Two co-infecting pathogens could likewise be competing to infect the same cells, which would result in identical clinical presentation as monotypic infection of the "winning" virus[104]. This has been described for a CHIKV/DENV co-infected patient, where the DENV replication was reported to be suppressed[131,135]. Finally, co-infecting pathogens could also have no impact on each other's replication or clinical presentation, as has been reported in CHIKV/DENV co-infections[126,129].

The number of reported co-infections with CHIKV, DENV and ZIKV is low. Furthermore, the limited evidence on the clinical significance of arboviral co-infections reveals a knowledge gap regarding

the prevalence of short- and/or long-term clinical presentation potentially caused by co-infection. In addition, the frequency of co-infections of CHIKV, DENV and ZIKV with co-circulating arboviruses or other infectious pathogens remains uninvestigated, largely because evaluating the co-infection frequency is challenging due to the dynamic background of mono-infection frequencies (i.e., the denominator for assessing co-infection frequency). This challenge of assessing mono-infection frequencies arises from the diagnostic difficulties in identifying acute CHIKV, DENV and ZIKV infections[108,126]. Finally, the impact of co-infection on a developing foetus in pregnancy remains unknown.

### 1.10 Arboviruses in pregnancy: CHIKV, DENV and ZIKV

Arboviruses infections in pregnancy expose pregnant women to various risks. Such risks can include more severe infection in pregnant women than in the general adult population, as during pregnancy several pathophysiological changes and immune adaptations occur to accommodate the foetus[136]. Thus, in pregnant women an arbovirus infection may lead to a more severe clinical presentation or even death[85]. Additionally, there is a risk of pregnant women transmitting the arbovirus to their foetus (i.e., antepartum mother-to-child transmission), which could lead to a risk of miscarriage (i.e., foetal loss before 28 weeks of gestation), stillbirth (i.e., foetal loss at 28 weeks of gestation or later), intrauterine growth restriction (IUGR) and/or a teratogenic effect on the foetus. Furthermore, women infected during late pregnancy risk transmitting the arbovirus close to birth or during delivery of the foetus (i.e., peripartum/ intrapartum mother-to-child transmission), which could result in severe neonatal infection. To date, limited data are available for CHIKV, DENV, and ZIKV infections in pregnancy, and each virus seems to impact the health of mother and foetus when infected in pregnancy differently (Table 2).

CHIKV antepartum (i.e., >7 days prior-to-delivery) and peripartum (i.e., 7 days to 3 days prior-to-delivery) mother-to-child transmission (MTCT) have been described[84]. A systematic review including a meta-analysis by Contopoulos-Ioannidis and colleagues found that the overall pooled risk of MTCT of 1331 CHIKV infections was 12.6% (95%CI: 13.6%-17.5%) and among 46 intrapartum maternal infections, defined as two days prior to delivery to two days post-delivery, was 50.3% (95%C:

34.9%-65.1%)[84]. Additionally, the review found no increased risk of miscarriages associated with CHIKV infections and no increase in the number of stillbirths, prematurity, or congenital malformations[84]. Nevertheless, the overall pooled-risk from 8 studies of symptomatic neonatal disease among maternal CHIKV infected women during gestation was 11.9% (95%CI: 3.9%-19.9%) and among intrapartum maternal infection from 3 studies was 50.3% (95%C: 3.8%-96.9%)[66,137]. Symptomatic infected newborns from maternal infections during gestation usually developed symptoms during their first week of life, but not at the time of birth. Commonly reported signs and symptoms included fever, diffuse limb edema, irritability, poor feeding, painful syndrome and rashes; occasionally, additional symptoms include sepsis-like syndrome with multiple organ involvement, meningoencephalitis with brain MRI abnormalities and sometimes even long term neurodevelopmental delays and devastating neurologic outcomes such as cerebral palsy[84].

In contrast to CHIKV, DENV has been described to cause an increased risk of severe disease in pregnant women in comparison to non-pregnant women, leading to DHF and DSS (OR 3.4, 95%CI: 2.1-5.4)[70,138,139]. Mortality among pregnant women with DHF increased relative to non-pregnant women with DHF (maternal mortality ratio in the DENV exposed cohort was about 1020 per100 000 live births)[85,140]. Further, antepartum mother-to-child transmission of DENV has been documented and is associated with increased foetal loss in the first half of pregnancy[70-72]. A recent retrospective study using linkage data was conducted on more than 16 million live births exposed to DENV in pregnancy from Brazil from 2006-2012[141]. The study suggests that DENV infection during pregnancy increases the odds of developing neurologic congenital anomalies by 50% and leads to a 4-fold increase for other congenital malformations of the brain, providing new evidence of an association of antenatal DENV infection in pregnancy with congenital anomalies of the brain[141]. Additionally, consequences of peripartum DENV mother-to-child transmission have been reported to cause severe neonatal infection with sepsis-like symptoms and acute respiratory distress[73,74].

Antepartum ZIKV mother-to-child transmission has been reported to be associated with foetal death[86]. Further, antepartum ZIKV mother-to-child transmission has been associated with foetal

*Table 2:* ***CHIKV, DENV and ZIKV in pregnancy.*** *Adapted from Charlier et al. (*2017)[10]**.**

| | CHIKV | DENV | ZIKV |
|---|---|---|---|
| **Maternal risk of infection in pregnancy** | | | |
| | **=** | **+**<br>Risk of severe infection,<br>**+**<br>risk of DHF/DSS [85] | **=** |
| **Consequences of antepartum mother-to-child transmission** | | | |
| Transmission | Documented, low incidence[84] | Documented[72] | Documented[142] |
| Miscarriage[e] | **=** [84] | **+**[72] | **(+)**[86] |
| Stillbirth[b] | **=** [143] | **(+)**[144] | **(+)**[86] |
| Preterm birth | **=** [143] | **+**[72] | Documented[86] |
| Low birthweight | **=** [143] | **+**[72] | n/a |
| Malformations | **=** | **+**[141]<br>Malformation of spinal cord (OR 5.4, 95% CI 1.0–26.9), Microcephaly (OR 1.7, 95% CI 0.33–8.32), | **+**[145,146]<br>Teratogenic, incidence of brain abnormalities in 1-13%, Severe microcephaly and other brain lesions, retinal lesions |
| Impaired neurological development | **=**[84]<br>0% (0/712) | **+**[141] | **+**[147-149]<br>Impaired neurological development and poor cranial growth, Irritability, pyramidal or extrapyramidal symptoms, epilepsy, dysphagia |
| **Consequences of peripartum mother-to-child transmission** | | | |
| Transmission | Documented[84], peripartum transmission rate 50% (95%CI: 34.90%-65.10%; 23/46) | Documented[74], Incidence unknown | Documented[150], rare |
| Consequences | **+**[84,151]<br>Neonatal symptomatic infections 50% (95% CIs: 34.90%-65.10%; 23/46), Severe long-term neurodevelopmental delays[c] | **+**[73,74]<br>Severe neonatal infection with sepsis-like symptoms and acute respiratory distress reported in case reports | **=**[150]<br>One asymptomatic and one case with mild rash (case reports from French Polynesia) |
| Neonatal death | 2.8% (95% CIs: 0.90%-6.29%; 5/182)[84] | n/a | n/a |

**+** *increased,* ***(+)*** *possibly increased,* **=** *not increased,* ***n/a*** *no data available, [a]Miscarriages are foetal losses before 28 weeks of gestation. [b]Stillbirths are foetal losses at 28 weeks of gestation or later. [c]At ~2 years of age in 50% of symptomatic neonatal infections (12 with CHIKV-encephalopathy and 22 with mild/ moderate prostration).*

developmental defects and teratogenicity[142]. The highest risk period of maternal ZIKV infection for damage to the central nervous system (CNS) has been proposed to be the first trimester or at the start of the second trimester, while impact on foetal growth and development may continue to occur with maternal infection well into the third trimester[145,146,152]. Congenital Zika Syndrome (CZS) has been described by the United States Centers for Disease Control and Prevention (CDC) as having five unique features that can be used to differentially diagnose CZS from other congenital conditions. These are: i) severe microcephaly in which the skull has partially collapsed; ii) decreased brain tissue with a specific pattern of brain damage, including subcortical calcifications; iii) damage to the back of the eye, including macular scarring and focal pigmentary retinal mottling; iv) congenital contractures, such as clubfoot or arthrogryposis; and v) hypertonia restricting body movement soon after birth[153]. Moreover, impaired postnatal neurological development with poor cranial growth, irritability, pyramidal or extrapyramidal symptoms, as well as dysphagia and epilepsy have been reported[147-149]. Peripartum mother-to-child transmission of ZIKV has been rarely reported and seems to mainly cause asymptomatic or mild outcomes, displayed with neonatal rash[150].

## 1.11 Study justification

CHIKV, DENV and ZIKV are the arboviruses of current public health concern in Latin America[6]. This public health relevance is manifested by their growing prevalence and ongoing co-circulation over the past 20 years in Latin America, and the continuing lack of optimal tools for prevention (e.g., vaccines) and treatment of infections (e.g., antivirals). CHIKV, DENV and ZIKV cause similar clinical symptoms, which make diagnosis and subsequent disease supportive care and prognosis a difficult challenge. This difficulty has potential implications for the health outcomes of patients and pregnancy.

The co-circulation of arboviruses in tropical and subtropical areas has led to the likelihood of co-infection occurring with arboviruses and other infectious diseases prevalent in these areas. Co-infected patients can present with similar clinical manifestations to monotypic infected patients, which complicates differential diagnosis[129,154,155]. Additionally, the actual influence of co-infections on the clinical presentation of respective arbovirus infections remains unstudied. Therefore, the frequency of co-infection occurrences and their impact on the clinical presentation of arboviruses should be assessed. In my research I chose to focus on concurrent co-infections of ZIKV infections. This is because a different systematic review identified no clinical significance on either symptoms or clinical outcomes of DENV/CHIKV co-infection. Moreover, ZIKV caused the largest arbovirus outbreak from 2015-2017 in Latin America, and in contrast to DENV and CHIKV infections, the short period of global ZIKV research has not yet evaluated the clinical significance of ZIKV co-infection.

An additional key concern regarding the setting of arbovirus co-circulation is the accurate identification of the specific arbovirus infections and co-infections. This is a particular challenge as the mild clinical presentation of CHIKV, DENV and ZIKV has been described to be similar[156]. All three arboviruses present with rash, fever, myalgia, arthralgia, conjunctivitis and headache. However, to date frequencies of signs and symptoms are unspecified[59,157]. As molecular testing was unavailable for DENV infections (i.e. PCR testing for the detection of DENV virus), serology of DENV infections was explored, but due to the high observed cross-reactivity between the flaviviruses DENV and ZIKV in serological testing (e.g. for the CDC MAC-ELISA for DENV IgM)[106], DENV infections have been excluded from the descriptive study of this MPhil. Until now, most CHIKV, DENV and ZIKV

frequencies of clinical signs and symptoms have been described in isolation from each other[15,50,86,158-160]. Furthermore, to our knowledge only three studies have reported the clinical presentation of CHIKV infections alongside ZIKV infections. However, these studies suffer from limitations of quality data and lack of explanation of statistical and diagnostic methods used[161-163].

Nevertheless, an accurate differential diagnosis of ZIKV and CHIKV infections is fundamentally important, as complications differ strongly between them. In adults and children, ZIKV infection has mainly been associated with the development of neurological complications, such as GBS, while CHIKV infection has been associated with neurological complications as well as persistent, disabling severe arthralgia[59,157,164]. Regarding mother-to-child transmission, not only have maternal ZIKV infections during pregnancy been confirmed to cause adverse birth outcomes, such as microcephaly, but maternal CHIKV infection around birth have also been reported to lead to severe long-term neurodevelopmental delays[60]. These known complications and long-term sequelae of ZIKV and CHIKV infections are becoming increasingly recognized and can lead to severe morbidity.

The co-circulation of CHIKV and ZIKV requires research. Hence, I chose to characterize the co-circulation of CHIKV and ZIKV within a cohort of pregnant women that presented with rash from 2015 to 2017 in Recife, Pernambuco, Brazil and investigate whether the symptom frequencies of the clinical presentation between ZIKV and CHIKV infections differ from each other[108]. To note, evidence displays that the clinical presentation of ZIKV and CHIKV infections are very similar in the general population and in pregnant women[50,136,165-170]. Additionally, the study of pregnant women in this context is highly relevant as they represent a sub-group especially at risk of serious complications.

To summarize, this study was conducted under the hypothesis that the differentiation of the respective arbovirus infections at the stage of symptom presentation could potentially facilitate clinical diagnosis. As CHIKV, DENV and ZIKV predominantly circulate in low-income settings and arbovirus laboratory testing is costly and time consuming, differentiating arbovirus infections upon symptom presentation would not only enable health care workers without access to laboratory testing to diagnose the origin of infection, it would also help them to diagnose the respective infection early after symptom onset. The advantages of an early diagnosis of infection after symptom onset is the potential

of an early initiation of appropriate clinical management and required follow up, which would reduce arbovirus infection associated complications and also remove potential strain from the health system. In addition, diagnosing an arbovirus infection upon symptom presentation will also guide laboratory testing by narrowing down the pathogens to be tested for, which is also of high relevance if resources for testing are limited. Furthermore, the consequences of early diagnosis, reducing testing, timely intervention and thus lowering numbers of arbovirus infection associated complications would relieve the public health services both financially and capacity-wise.

Taken together, my systematic review and my descriptive study of this MPhil research project aim to contribute to what is known on co-circulating arboviruses in Latin America by contributing to fill the gap of knowledge on clinical significance of ZIKV co-infections and evaluating the clinical presentation of CHIKV and ZIKV infections. Overall, this study aims to improve preparedness for future arbovirus outbreaks in a time of ongoing arbovirus co-circulation and continuing unavailability of licenced antivirals or vaccines.

## 1.12 References


1 Wilder-Smith, A. *et al.* Epidemic arboviral diseases: priorities for research and public health. *The Lancet. Infectious diseases* **17**, e101-e106, doi:10.1016/s1473-3099(16)30518-7 (2017).

2 Murray, C. J. *et al.* Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: quantifying the epidemiological transition. *Lancet* **386**, 2145-2191, doi:10.1016/s0140-6736(15)61340-x (2015).

3 Gubler, D. J. & Clark, G. G. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. *Emerging infectious diseases* **1**, 55-57, doi:10.3201/eid0102.952004 (1995).

4 Braack, L., Gouveia de Almeida, A. P., Cornel, A. J., Swanepoel, R. & de Jager, C. Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites & vectors* **11**, 29, doi:10.1186/s13071-017-2559-9 (2018).

5 Hotez, P. J. & Murray, K. O. Dengue, West Nile virus, chikungunya, Zika-and now Mayaro? *PLoS neglected tropical diseases* **11**, e0005462, doi:10.1371/journal.pntd.0005462 (2017).

6 Vasconcelos, P. F. & Calisher, C. H. Emergence of Human Arboviral Diseases in the Americas, 2000-2016. *Vector borne and zoonotic diseases (Larchmont, N.Y.)* **16**, 295-301, doi:10.1089/vbz.2016.1952 (2016).

7 Zellweger, R. M. *et al.* Socioeconomic and environmental determinants of dengue transmission in an urban setting: An ecological study in Nouméa, New Caledonia. *PLoS neglected tropical diseases* **11**, e0005471-e0005471, doi:10.1371/journal.pntd.0005471 (2017).

8 Braga, C. *et al.* Seroprevalence and risk factors for dengue infection in socio-economically distinct areas of Recife, Brazil. *Acta tropica* **113**, 234-240, doi:10.1016/j.actatropica.2009.10.021 (2010).

9 Alatoom, A. & Payne, D. An Overview of Arboviruses and Bunyaviruses. *Laboratory Medicine* **40**, 237-240, doi:10.1309/LMPX9OEOAOPPBCJH %J Laboratory Medicine (2009).

10 Charlier, C., Beaudoin, M. C., Couderc, T., Lortholary, O. & Lecuit, M. Arboviruses and pregnancy: maternal, fetal, and neonatal effects. *The Lancet. Child & adolescent health* **1**, 134-146, doi:10.1016/s2352-4642(17)30021-4 (2017).

11 Weaver, S. C. & Reisen, W. K. Present and future arboviral threats. *Antiviral research* **85**, 328-345, doi:10.1016/j.antiviral.2009.10.008 (2010).

12 Gould, E., Pettersson, J., Higgs, S., Charrel, R. & de Lamballerie, X. Emerging arboviruses: Why today? *One health (Amsterdam, Netherlands)* **4**, 1-13, doi:10.1016/j.onehlt.2017.06.001 (2017).

13 Lim, S. K., Lim, J. K. & Yoon, I. K. An Update on Zika Virus in Asia. *Infection & chemotherapy* **49**, 91-100, doi:10.3947/ic.2017.49.2.91 (2017).

14 Kilpatrick, A. M. & Randolph, S. E. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet (London, England)* **380**, 1946-1955, doi:10.1016/S0140-6736(12)61151-9 (2012).

15 Anyamba, A. *et al.* Global Disease Outbreaks Associated with the 2015-2016 El Niño Event. *Scientific reports* **9**, 1930-1930, doi:10.1038/s41598-018-38034-z (2019).

16 Lumsden, W. H. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. II. General description and epidemiology. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **49**, 33-57 (1955).

17 Peper, S. M., Monson, B. J., Van Schooneveld, T. & Smith, C. J. That Which Bends Up: A Case Report and Literature Review of Chikungunya Virus. *Journal of general internal medicine* **31**, 576-581, doi:10.1007/s11606-015-3459-3 (2016).

18 Wahid, B., Ali, A., Rafique, S. & Idrees, M. Global expansion of chikungunya virus: mapping the 64-year history. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* **58**, 69-76, doi:10.1016/j.ijid.2017.03.006 (2017).

19 Weaver, S. C. & Lecuit, M. Chikungunya virus and the global spread of a mosquito-borne disease. *The New England journal of medicine* **372**, 1231-1239, doi:10.1056/NEJMra1406035 (2015).

20 Olajiga, O. M. *et al.* Chikungunya Virus Seroprevalence and Associated Factors among Hospital Attendees in Two States of Southwest Nigeria: A Preliminary Assessment. *Immunological investigations* **46**, 552-565, doi:10.1080/08820139.2017.1319383 (2017).

21 LaBeaud, A. D. *et al.* High rates of o'nyong nyong and Chikungunya virus transmission in coastal Kenya. *PLoS neglected tropical diseases* **9**, e0003436, doi:10.1371/journal.pntd.0003436 (2015).

22 Afreen, N. *et al.* Molecular characterization of dengue and chikungunya virus strains circulating in New Delhi, India. *Microbiology and immunology* **58**, 688-696, doi:10.1111/1348-0421.12209 (2014).

23 Khatun, S. *et al.* An Outbreak of Chikungunya in Rural Bangladesh, 2011. *PLoS neglected tropical diseases* **9**, e0003907, doi:10.1371/journal.pntd.0003907 (2015).

24 Crosby, L. *et al.* Severe manifestations of chikungunya virus in critically ill patients during the 2013-2014 Caribbean outbreak. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* **48**, 78-80, doi:10.1016/j.ijid.2016.05.010 (2016).

25 Hamer, D. H. & Chen, L. H. Chikungunya: establishing a new home in the Western hemisphere. *Annals of internal medicine* **161**, 827-828, doi:10.7326/m14-1958 (2014).

26 Delisle, E. *et al.* Chikungunya outbreak in Montpellier, France, September to October 2014. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* **20** (2015).

27 Gould, E. A., Gallian, P., De Lamballerie, X. & Charrel, R. N. First cases of autochthonous dengue fever and chikungunya fever in France: from bad dream to reality! *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **16**, 1702-1704, doi:10.1111/j.1469-0691.2010.03386.x (2010).

28 Tomasello, D. & Schlagenhauf, P. Chikungunya and dengue autochthonous cases in Europe, 2007-2012. *Travel medicine and infectious disease* **11**, 274-284, doi:10.1016/j.tmaid.2013.07.006 (2013).

29 Rezza, G. *et al.* Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet* **370**, 1840-1846, doi:10.1016/s0140-6736(07)61779-6 (2007).

30 Enserink, M. Infectious diseases. Chikungunya: no longer a third world disease. *Science* **318**, 1860-1861, doi:10.1126/science.318.5858.1860 (2007).

31 Nsoesie, E. O. *et al.* Global distribution and environmental suitability for chikungunya virus, 1952 to 2015. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* **21**, doi:10.2807/1560-7917.Es.2016.21.20.30234 (2016).

32 Halstead SB. Dengue: overview and history. *Tropical Medicine: Science and Practice. London: Imperial College Press;* (2008).

33 Halstead, S. B. Reappearance of chikungunya, formerly called dengue, in the Americas. *Emerging infectious diseases* **21**, 557-561, doi:10.3201/eid2104.141723 (2015).

34 Hotta, S. Experimental studies on dengue. I. Isolation, identification and modification of the virus. *The Journal of infectious diseases* **90**, 1-9 (1952).

35 Messina, J. P. *et al.* Global spread of dengue virus types: mapping the 70 year history. *Trends in microbiology* **22**, 138-146, doi:10.1016/j.tim.2013.12.011 (2014).

36 Gubler, D. J. Dengue and dengue hemorrhagic fever. *Clinical microbiology reviews* **11**, 480-496 (1998).

37 Gubler, D. J. Aedes aegypti and Aedes aegypti-borne disease control in the 1990s: top down or bottom up. Charles Franklin Craig Lecture. *The American journal of tropical medicine and hygiene* **40**, 571-578 (1989).

38 Schliessman DJ, C. L. A review of the status of yellow fever and Aedes aegypti eradication programs in the Americas. *Mosquito News* **34** (1974).

39 Brady, O. J. *et al.* Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS neglected tropical diseases* **6**, e1760, doi:10.1371/journal.pntd.0001760 (2012).

40 Bhatt, S. *et al.* The global distribution and burden of dengue. *Nature* **496**, 504-507, doi:10.1038/nature12060 (2013).

41 Dick, G. W. Zika virus. II. Pathogenicity and physical properties. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **46**, 521-534 (1952).

42 Dick, G. W., Kitchen, S. F. & Haddow, A. J. Zika virus. I. Isolations and serological specificity. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **46**, 509-520 (1952).

43 Smithburn, K. C. Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa. *Journal of immunology (Baltimore, Md. : 1950)* **69**, 223-234 (1952).

44 Macnamara, F. N. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **48**, 139-145 (1954).

45 Haddow, A. D. *et al.* Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. *PLoS neglected tropical diseases* **6**, e1477, doi:10.1371/journal.pntd.0001477 (2012).

46 Simpson, D. I. ZIKA VIRUS INFECTION IN MAN. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **58**, 335-338 (1964).

47 Olson, J. G., Ksiazek, T. G., Suhandiman & Triwibowo. Zika virus, a cause of fever in Central Java, Indonesia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 389-393 (1981).

48 Fagbami, A. H. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. *The Journal of hygiene* **83**, 213-219 (1979).

49 Lowe, R. *et al.* The Zika Virus Epidemic in Brazil: From Discovery to Future Implications. *International journal of environmental research and public health* **15**, 96, doi:10.3390/ijerph15010096 (2018).

50 Duffy, M. R. *et al.* Zika Virus Outbreak on Yap Island, Federated States of Micronesia. *New England Journal of Medicine* **360**, 2536-2543, doi:10.1056/NEJMoa0805715 (2009).

51 Musso, D. *et al.* Zika virus in French Polynesia 2013-14: anatomy of a completed outbreak. *The Lancet. Infectious diseases* **18**, e172-e182, doi:10.1016/s1473-3099(17)30446-2 (2018).

52 Lanciotti, R. S., Lambert, A. J., Holodniy, M., Saavedra, S. & Signor Ldel, C. Phylogeny of Zika Virus in Western Hemisphere, 2015. *Emerg Infect Dis* **22**, 933-935, doi:10.3201/eid2205.160065 (2016).

53 Osterlund, P. *et al.* Asian and African lineage Zika viruses show differential replication and innate immune responses in human dendritic cells and macrophages. *Scientific reports* **9**, 15710, doi:10.1038/s41598-019-52307-1 (2019).

54 Lanciotti, R. S. *et al.* Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* **14**, 1232-1239, doi:10.3201/eid1408.080287 (2008).

55 Cao-Lormeau, V. M. *et al.* Zika virus, French polynesia, South pacific, 2013. *Emerg Infect Dis* **20**, 1085-1086, doi:10.3201/eid2006.140138 (2014).

56 Hennessey, M., Fischer, M. & Staples, J. E. Zika Virus Spreads to New Areas - Region of the Americas, May 2015-January 2016. *MMWR. Morbidity and mortality weekly report* **65**, 55-58, doi:10.15585/mmwr.mm6503e1 (2016).

57 Faria, N. R. *et al.* Zika virus in the Americas: Early epidemiological and genetic findings. *Science (New York, N.Y.)* **352**, 345-349, doi:10.1126/science.aaf5036 (2016).

58 World Health Organisation. ZIKA SITUATION REPORT *[*http://apps.who.int/iris/bitstream/10665/204348/1/zikasitrep_5Feb2016_eng.pdf?ua=1*]* (2016).

59 World Health Organisation. Zika Virus Fact sheet, doi:http://www.who.int/news-room/fact-sheets/detail/zika-virus (2018).

60 World Health Organisation. Zika epidemiology update https://www.who.int/emergencies/diseases/zika/zika-epidemiology-update-july-2019.pdf?ua=1 (2019).

61 Ministério da Saúde Secretaria de Vigilância em Saúde—Ministério da Saúde monitoramento dos casos de dengue, f. d. c. e. f. p. v. Z. a. a. s. e., 2016. Bol. Epidemiol. 2017;48:1–11.

62 Kraemer, M. U. G. *et al.* The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. *eLife* **4**, e08347-e08347, doi:10.7554/eLife.08347 (2015).

63 Louten, J. in *Essential Human Virology* (ed Jennifer Louten) 291-310 (Academic Press, 2016).

64 Petersen, L. R., Stramer, S. L. & Powers, A. M. Chikungunya virus: possible impact on transfusion medicine. *Transfusion medicine reviews* **24**, 15-21, doi:10.1016/j.tmrv.2009.09.002 (2010).

65 Gregory, C. J. *et al.* Modes of Transmission of Zika Virus. *The Journal of infectious diseases* **216**, S875-s883, doi:10.1093/infdis/jix396 (2017).

66 Gerardin, P. *et al.* Multidisciplinary prospective study of mother-to-child chikungunya virus infections on the island of La Reunion. *PLoS medicine* **5**, e60, doi:10.1371/journal.pmed.0050060 (2008).

67 Nair, P. M. Chikungunya in neonates. *Indian pediatrics* **45**, 605 (2008).

68 Rao, G., Khan, Y. Z. & Chitnis, D. S. Chikungunya infection in neonates. *Indian pediatrics* **45**, 240-242 (2008).

69 Shenoy, S. & Pradeep, G. C. Neurodevelopmental outcome of neonates with vertically transmitted Chikungunya fever with encephalopathy. *Indian pediatrics* **49**, 238-240 (2012).

70 Carles, G., Talarmin, A., Peneau, C. & Bertsch, M. Dengue fever and pregnancy. A study of 38 cases in french Guiana. *Journal de gynecologie, obstetrique et biologie de la reproduction* **29**, 758-762 (2000).

71 Tan, P. C. *et al.* Dengue infection and miscarriage: a prospective case control study. *PLoS neglected tropical diseases* **6**, e1637, doi:10.1371/journal.pntd.0001637 (2012).

72 Paixao, E. S., Teixeira, M. G., Costa, M. & Rodrigues, L. C. Dengue during pregnancy and adverse fetal outcomes: a systematic review and meta-analysis. *The Lancet. Infectious diseases* **16**, 857-865, doi:10.1016/s1473-3099(16)00088-8 (2016).

73 Sirinavin, S. *et al.* Vertical dengue infection: case reports and review. *The Pediatric infectious disease journal* **23**, 1042-1047 (2004).

74 Basurko, C., Carles, G., Youssef, M. & Guindi, W. E. Maternal and fetal consequences of dengue fever during pregnancy. *European journal of obstetrics, gynecology, and reproductive biology* **147**, 29-32, doi:10.1016/j.ejogrb.2009.06.028 (2009).

75 Schaefer TJ, W. R. Dengue Fever. . (2019).

76 Centers for Disease Control and Prevention. Chikungunya - Chapter 3 - 2018 Yellow Book | Travelers' Health | CDC. *CDC website:* https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/chikungunya (2018).

77 Haby, M. M., Pinart, M., Elias, V. & Reveiz, L. Prevalence of asymptomatic Zika virus infection: a systematic review. *Bulletin of the World Health Organization* **96**, 402-413D, doi:10.2471/BLT.17.201541 (2018).

78 Dias, J. *et al.* Seroprevalence of Chikungunya Virus after Its Emergence in Brazil. *Emerging infectious diseases* **24**, 617-624, doi:10.3201/eid2404.171370 (2018).

79 Beltrán-Silva, S. L., Chacón-Hernández, S. S., Moreno-Palacios, E. & Pereyra-Molina, J. Á. *Clinical and differential diagnosis: Dengue, chikungunya and Zika*. Vol. 81 (2016).

80 Sheraz, F., Tahir, H., Saqi, J. & Daruwalla, V. Dengue Fever Presenting Atypically with Viral Conjunctivitis and Subacute Thyroiditis. *Journal of the College of Physicians and Surgeons--Pakistan : JCPSP* **26**, S33-34 (2016).

81 Katzelnick, L. C. *et al.* Antibody-dependent enhancement of severe dengue disease in humans. *Science* **358**, 929, doi:10.1126/science.aan6836 (2017).

82 Kalayanarooj, S. Clinical Manifestations and Management of Dengue/DHF/DSS. *Tropical medicine and health* **39**, 83-87, doi:10.2149/tmh.2011-S10 (2011).

83 Black, W. C. t. *et al.* Flavivirus susceptibility in Aedes aegypti. *Archives of medical research* **33**, 379-388 (2002).

84 Contopoulos-Ioannidis, D., Newman-Lindsay, S., Chow, C. & LaBeaud, A. D. Mother-to-child transmission of Chikungunya virus: A systematic review and meta-analysis. *PLoS neglected tropical diseases* **12**, e0006510, doi:10.1371/journal.pntd.0006510 (2018).

85 Paixao, E. S. *et al.* Dengue in pregnancy and maternal mortality: a cohort analysis using routine data. *Scientific reports* **8**, 9938, doi:10.1038/s41598-018-28387-w (2018).

86 Brasil, P. *et al.* Zika Virus Infection in Pregnant Women in Rio de Janeiro. *The New England journal of medicine* **375**, 2321-2334, doi:10.1056/NEJMoa1602412 (2016).

87 Noronha, L., Zanluca, C., Azevedo, M. L., Luz, K. G. & Santos, C. N. Zika virus damages the human placental barrier and presents marked fetal neurotropism. *Memorias do Instituto Oswaldo Cruz* **111**, 287-293, doi:10.1590/0074-02760160085 (2016).

88 Cao-Lormeau, V. M. *et al.* Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* **387**, 1531-1539, doi:10.1016/s0140-6736(16)00562-6 (2016).

89 World Health, O. Guillain-Barré syndrome—Colombia and Venezuela. . (2016).

90 Pomar, L. *et al.* Maternal-fetal transmission and adverse perinatal outcomes in pregnant women infected with Zika virus: prospective cohort study in French Guiana. **363**, k4431, doi:10.1136/bmj.k4431 %J BMJ (2018).

91 Hoen, B. *et al.* Pregnancy Outcomes after ZIKV Infection in French Territories in the Americas. **378**, 985-994, doi:10.1056/NEJMoa1709481 (2018).

92 Reynolds MR, J. A., Petersen EE, et al. . Vital Signs: Update on Zika Virus–Associated Birth Defects and Evaluation of All U.S. Infants with Congenital Zika Virus Exposure *U.S. Zika Pregnancy Registry, 2016. MMWR Morb Mortal Wkly Rep* doi:DOI: http://dx.doi.org/10.15585/mmwr.mm6613e1. (2017;).

93 Nogueira, M. L. *et al.* Adverse birth outcomes associated with Zika virus exposure during pregnancy in Sao Jose do Rio Preto, Brazil. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **24**, 646-652, doi:10.1016/j.cmi.2017.11.004 (2018).

94 Euroimmune. https://www.euroimmun.com/products/indications/infektions-serologie/zika-viruses.html. (2018).

95 Atkinson, B. *et al.* Detection of Zika Virus in Semen. *Emerging infectious diseases* **22**, 940-940, doi:10.3201/eid2205.160107 (2016).

96 Gourinat, A. C., O'Connor, O., Calvez, E., Goarant, C. & Dupont-Rouzeyrol, M. Detection of Zika virus in urine. *Emerg Infect Dis* **21**, 84-86, doi:10.3201/eid2101.140894 (2015).

97 World Health Organisation. Laboratory testing : https://www.who.int/csr/resources/publications/zika/laboratory-testing/en/. (2016).

98 Musso, D. *et al.* Detection of chikungunya virus in saliva and urine. *Virology journal* **13**, 102, doi:10.1186/s12985-016-0556-9 (2016).

99 Hirayama, T. *et al.* Detection of dengue virus genome in urine by real-time reverse transcriptase PCR: a laboratory diagnostic method useful after disappearance of the genome in serum. *J Clin Microbiol* **50**, 2047-2052, doi:10.1128/JCM.06557-11 (2012).

100 Halstead, S. B. Etiologies of the experimental dengues of Siler and Simmons. *The American journal of tropical medicine and hygiene* **23**, 974-982, doi:10.4269/ajtmh.1974.23.974 (1974).

101 Galán-Huerta, K. A., Rivas-Estilla, A. M., Fernández-Salas, I., Farfan-Ale, J. A. & Ramos-Jiménez, J. Chikungunya virus: A general overview. *Medicina Universitaria* **17**, 175-183, doi:10.1016/j.rmu.2015.06.001 (2015).

102 Charrel, R. N. *et al.* Background review for diagnostic test development for Zika virus infection. *Bull World Health Organ* **94**, 574-584d, doi:10.2471/blt.16.171207 (2016).

103 Wilson, H. L., Tran, T., Druce, J., Dupont-Rouzeyrol, M. & Catton, M. Neutralization Assay for Zika and Dengue Viruses by Use of Real-Time-PCR-Based Endpoint Assessment. *J Clin Microbiol* **55**, 3104-3112, doi:10.1128/JCM.00673-17 (2017).

104 Roehrig, J. T., Hombach, J. & Barrett, A. D. Guidelines for Plaque-Reduction Neutralization Testing of Human Antibodies to Dengue Viruses. *Viral immunology* **21**, 123-132, doi:10.1089/vim.2008.0007 (2008).

105 Azami, N. A., Moi, M. L. & Takasaki, T. Neutralization Assay for Chikungunya Virus Infection: Plaque Reduction Neutralization Test. *Methods in molecular biology (Clifton, N.J.)* **1426**, 273-282, doi:10.1007/978-1-4939-3618-2_25 (2016).

106 Wilder-Smith, A. *et al.* Zika vaccines and therapeutics: landscape analysis and challenges ahead. *BMC Medicine* **16**, 84, doi:10.1186/s12916-018-1067-x (2018).

107 Barzon, L. *et al.* Virus and Antibody Dynamics in Travelers With Acute Zika Virus Infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **66**, 1173-1180, doi:10.1093/cid/cix967 (2018).

108 Ximenes, R. A. d. A. *et al.* Zika virus infection in pregnancy: Establishing a case definition for clinical research on pregnant women with rash in an active transmission setting. *PLoS neglected tropical diseases* **13**, e0007763, doi:10.1371/journal.pntd.0007763 (2019).

109 Centers for Disease Control and Prevention. Dengue virus: Symptoms and treatment. *CDC website:* https://www.cdc.gov/dengue/symptoms/index.html (2019).

110 Centers for Disease Control and Prevention. Chikungunya virus: Symptoms, Diagnosis & Treatment. *CDC website:* https://www.cdc.gov/chikungunya/symptoms/index.html (2018).

111 Centers for Disease Control and Prevention. Chikungunya virus: atypical and severe disease symptoms. *CDC website:* https://www.cdc.gov/chikungunya/pdfs/Chikungunya-atypical-severe-disease_Healthcare-provider-factsheet-10-07-2014.pdf (2014).

112 Centers for Disease Control and Prevention. Zika virus: treatment. *CDC website:* https://www.cdc.gov/zika/symptoms/treatment.html (2019).

113 Abbink, P., Stephenson, K. E. & Barouch, D. H. Zika virus vaccines. *Nat Rev Microbiol* **16**, 594-600, doi:10.1038/s41579-018-0039-7 (2018).

114 Fernandez, E. & Diamond, M. S. Vaccination strategies against Zika virus. *Curr Opin Virol* **23**, 59-67, doi:10.1016/j.coviro.2017.03.006 (2017).

115 NIH: National Institute of Allergy and Infectious Diseases. Zika Virus Vaccines www.niaid.nih.gov.

116 Plante, K. *et al.* Novel chikungunya vaccine candidate with an IRES-based attenuation and host range alteration mechanism. *PLoS pathogens* **7**, e1002142, doi:10.1371/journal.ppat.1002142 (2011).

117 Hallengard, D. *et al.* Novel attenuated Chikungunya vaccine candidates elicit protective immunity in C57BL/6 mice. *Journal of virology* **88**, 2858-2866, doi:10.1128/jvi.03453-13 (2014).

118 Schwameis, M., Buchtele, N., Wadowski, P. P., Schoergenhofer, C. & Jilma, B. Chikungunya vaccines in development. *Hum Vaccin Immunother* **12**, 716-731, doi:10.1080/21645515.2015.1101197 (2016).

119 U.S. Food & Drugs Administration. First FDA-approved vaccine for the prevention of dengue disease in endemic regionsFirst FDA-approved vaccine for the prevention of dengue disease in endemic regions. https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-dengue-disease-endemic-regions (2019).

120 Centers for Disease Control and Prevention. Dengue Vaccine. *CDC website:* https://www.cdc.gov/dengue/prevention/dengue-vaccine.html (2019).

121 Gubler, D. J. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends in microbiology* **10**, 100-103, doi:10.1016/s0966-842x(01)02288-0 (2002).

122 Deng, S. Q. *et al.* A Review on Dengue Vaccine Development. *Vaccines* **8**, doi:10.3390/vaccines8010063 (2020).

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

124 Ball, J. D. *et al.* Clinical and Epidemiologic Patterns of Chikungunya Virus Infection and Coincident Arboviral Disease in a School Cohort in Haiti, 2014-2015. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **68**, 919-926, doi:10.1093/cid/ciy582 (2019).

125 Laoprasopwattana, K., Kaewjungwad, L., Jarumanokul, R. & Geater, A. Differential diagnosis of Chikungunya, dengue viral infection and other acute febrile illnesses in children. *The Pediatric infectious disease journal* **31**, 459-463, doi:10.1097/INF.0b013e31824bb06d (2012).

126 Furuya-Kanamori, L. *et al.* Co-distribution and co-infection of chikungunya and dengue viruses. *BMC infectious diseases* **16**, 84-84, doi:10.1186/s12879-016-1417-2 (2016).

127 Schilling, S., Emmerich, P., Gunther, S. & Schmidt-Chanasit, J. Dengue and Chikungunya virus co-infection in a German traveller. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* **45**, 163-164, doi:10.1016/j.jcv.2009.04.001 (2009).

128 Omarjee, R. *et al.* Importance of case definition to monitor ongoing outbreak of chikungunya virus on a background of actively circulating dengue virus, St Martin, December 2013 to January 2014. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* **19** (2014).

129 Taraphdar, D., Sarkar, A., Mukhopadhyay, B. B. & Chatterjee, S. A comparative study of clinical features between monotypic and dual infection cases with Chikungunya virus and dengue virus in West Bengal, India. *The American journal of tropical medicine and hygiene* **86**, 720-723, doi:10.4269/ajtmh.2012.11-0704 (2012).

130 Chahar, H. S. *et al.* Co-infections with chikungunya virus and dengue virus in Delhi, India. *Emerg Infect Dis* **15**, 1077-1080, doi:10.3201/eid1507.080638 (2009).

131 Vogels, C. B. F. *et al.* Arbovirus coinfection and co-transmission: A neglected public health concern? *PLoS biology* **17**, e3000130, doi:10.1371/journal.pbio.3000130 (2019).

132 Hollidge, B. S., Weiss, S. R. & Soldan, S. S. The role of interferon antagonist, non-structural proteins in the pathogenesis and emergence of arboviruses. *Viruses* **3**, 629-658, doi:10.3390/v3060629 (2011).

133 Moon, S. L. *et al.* A noncoding RNA produced by arthropod-borne flaviviruses inhibits the cellular exoribonuclease XRN1 and alters host mRNA stability. *RNA (New York, N.Y.)* **18**, 2029-2040, doi:10.1261/rna.034330.112 (2012).

134 Beatty, P. R. *et al.* Dengue virus NS1 triggers endothelial permeability and vascular leak that is prevented by NS1 vaccination. *Science translational medicine* **7**, 304ra141, doi:10.1126/scitranslmed.aaa3787 (2015).

135 Zaidi, M. B. *et al.* Competitive suppression of dengue virus replication occurs in chikungunya and dengue co-infected Mexican infants. *Parasites & vectors* **11**, 378, doi:10.1186/s13071-018-2942-1 (2018).

136 Kourtis, A. P., Read, J. S. & Jamieson, D. J. Pregnancy and infection. *N Engl J Med* **370**, 2211-2218, doi:10.1056/NEJMra1213566 (2014).

137 Senanayake MPSS, V. K., Gunassena S, Lamabadusurlya SP,. Vertical transmission Chikungunya infection. *Cylon Med J* **54(2):47–50** (2009).

138 Machado, C. R. *et al.* Is pregnancy associated with severe dengue? A review of data from the Rio de Janeiro surveillance information system. *PLoS neglected tropical diseases* **7**, e2217, doi:10.1371/journal.pntd.0002217 (2013).

139 Waduge, R. *et al.* Dengue infections during pregnancy: a case series from Sri Lanka and review of the literature. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* **37**, 27-33, doi:10.1016/j.jcv.2006.06.002 (2006).

140 Feitoza, H. A. C., Koifman, S., Koifman, R. J. & Saraceni, V. Dengue infection during pregnancy and adverse maternal, fetal, and infant health outcomes in Rio Branco, Acre State, Brazil, 2007-2012. *Cadernos de saude publica* **33**, e00178915, doi:10.1590/0102-311x00178915 (2017).

141 Paixão, E. S., Teixeira, M. G., Costa, M. d. C. N., Barreto, M. L. & Rodrigues, L. C. Symptomatic Dengue during Pregnancy and Congenital Neurologic Malformations. *Emerging infectious diseases* **24**, 1748-1750, doi:10.3201/eid2409.170361 (2018).

142 de Araujo, T. V. B. *et al.* Association between microcephaly, Zika virus infection, and other risk factors in Brazil: final report of a case-control study. *The Lancet. Infectious diseases* **18**, 328-336, doi:10.1016/s1473-3099(17)30727-2 (2018).

143 Xavier, F. *et al.* Chikungunya Virus Infection during Pregnancy, Réunion, France, 2006. *Emerging Infectious Disease journal* **16**, 418, doi:10.3201/eid1603.091403 (2010).

144 Friedman, E. E. *et al.* Symptomatic Dengue infection during pregnancy and infant outcomes: a retrospective cohort study. *PLoS neglected tropical diseases* **8**, e3226, doi:10.1371/journal.pntd.0003226 (2014).

145 Cauchemez, S. *et al.* Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study. *Lancet* **387**, 2125-2132, doi:10.1016/s0140-6736(16)00651-6 (2016).

146 Johansson, M. A., Mier-y-Teran-Romero, L., Reefhuis, J., Gilboa, S. M. & Hills, S. L. Zika and the Risk of Microcephaly. *The New England journal of medicine* **375**, 1-4, doi:10.1056/NEJMp1605367 (2016).

147 Silva, A. A. *et al. Early Growth and Neurologic Outcomes of Infants with Probable Congenital Zika Virus Syndrome*. Vol. 22 (2016).

148 Moura da Silva, A. A. *et al.* Early Growth and Neurologic Outcomes of Infants with Probable Congenital Zika Virus Syndrome. *Emerg Infect Dis* **22**, 1953-1956, doi:10.3201/eid2211.160956 (2016).

149 Schuler-Faccini, L. *et al.* Possible Association Between Zika Virus Infection and Microcephaly - Brazil, 2015. *MMWR. Morbidity and mortality weekly report* **65**, 59-62, doi:10.15585/mmwr.mm6503e2 (2016).

150 Besnard, M., Lastere, S., Teissier, A., Cao-Lormeau, V. & Musso, D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* **19** (2014).

151 Gerardin, P. *et al.* Neurocognitive outcome of children exposed to perinatal mother-to-child Chikungunya virus infection: the CHIMERE cohort study on Reunion Island. *PLoS neglected tropical diseases* **8**, e2996, doi:10.1371/journal.pntd.0002996 (2014).

152 De Santis, M., Cavaliere, A. F., Straface, G. & Caruso, A. Rubella infection in pregnancy. *Reproductive toxicology (Elmsford, N.Y.)* **21**, 390-398, doi:10.1016/j.reprotox.2005.01.014 (2006).

153 Centers for Disease Control and Prevention. Congenital Zika Syndrome & Other birt defects. *CDC website:* www.cdc.gov/pregnancy/zika/testing-follow-up/zika-syndrome-birth-defects.html (2019).

154 Nkoghe, D. *et al.* No clinical or biological difference between Chikungunya and Dengue Fever during the 2010 Gabonese outbreak. *Infectious disease reports* **4**, e5, doi:10.4081/idr.2012.e5 (2012).

155 Raut, C. G., Rao, N. M., Sinha, D. P., Hanumaiah, H. & Manjunatha, M. J. Chikungunya, dengue, and malaria co-infection after travel to Nigeria, India. *Emerging infectious diseases* **21**, 908-909, doi:10.3201/eid2105.141804 (2015).

156 Musso, D., Cao-Lormeau, V. M. & Gubler, D. J. Zika virus: following the path of dengue and chikungunya? *Lancet (London, England)* **386**, 243-244, doi:10.1016/s0140-6736(15)61273-9 (2015).

157 World Health Organisation. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. *World Health Organization. Geneva.* **1-147.** (2009).

158 Joob, B. & Wiwanitkit, V. Clinical relevance of Zika symptoms in the context of a Zika Dengue epidemic. *Journal of infection and public health* **13**, 158, doi:10.1016/j.jiph.2019.10.001 (2020).

159 van Genderen, F. T. *et al.* First Chikungunya Outbreak in Suriname; Clinical and Epidemiological Features. *PLoS Negl Trop Dis* **10**, e0004625, doi:10.1371/journal.pntd.0004625 (2016).

160 Guanche Garcell, H. *et al.* Clinical relevance of Zika symptoms in the context of a Zika Dengue epidemic. *Journal of infection and public health*, doi:10.1016/j.jiph.2019.07.006 (2019).

161 Ioos, S. *et al.* Current Zika virus epidemiology and recent epidemics. *Med Mal Infect* **44**, 302-307, doi:10.1016/j.medmal.2014.04.008 (2014).

162 Waggoner, J. J. *et al.* Viremia and Clinical Presentation in Nicaraguan Patients Infected With Zika Virus, Chikungunya Virus, and Dengue Virus. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **63**, 1584-1590, doi:10.1093/cid/ciw589 (2016).

163 Brasil. (Ministério da Saúde, Secretaria de Atenção à Saúde Brasília, DF, 2015).

164 World Health Organisation. Chikungunya virus: Fact sheet. . https://www.who.int/news-room/fact-sheets/detail/chikungunya (2017).

165 Mallet HP, V. A., Musso D. [Bilan de l'epidemie a virus Zika en polynesie francaise 2013-2014. Bises Bulletin D'informaion Sanitaires, Epidemiologiques Et Statistiques 2015]. . *French. [Accessed August 14, 2016].* (2016).

166 Zika virus infection: global update on epidemiology and potentially associated clinical manifestations. *Wkly Epidemiol Rec* **91**, 73-81 (2016).

167 Ceccaldi PF, L. P., Mandelbrot L. . Infections virales émergentes et grossesse. *Gynecol Obstet Fertil* **35: 339–342.** (2007).

168 Lin, H. Z., Tambyah, P. A., Yong, E. L., Biswas, A. & Chan, S.-Y. A review of Zika virus infections in pregnancy and implications for antenatal care in Singapore. *Singapore Med J* **58**, 171-178, doi:10.11622/smedj.2017026 (2017).

169 Pazos, M., Sperling, R. S., Moran, T. M. & Kraus, T. A. The influence of pregnancy on systemic immunity. *Immunol Res* **54**, 254-261, doi:10.1007/s12026-012-8303-9 (2012).

170 Aghaeepour, N. *et al.* An immune clock of human pregnancy. *Sci Immunol* **2**, doi:10.1126/sciimmunol.aan2946 (2017).

## 2. CHAPTER II: METHODS & RESULTS

This chapter contains two research papers that form the basis of my study. The first paper systematically reviews ZIKV co-infections, including assessing the co-infection frequency among ZIKV infected cases and the impact of co-infections on the clinical presentation of ZIKV infections (Paper I). The second paper demonstrates the temporal and geographical co-circulation of CHIKV and ZIKV in a cohort of pregnant women presenting with rash in Recife, Brazil from 2015 to 2017. Furthermore, the second paper also investigates whether CHIKV and ZIKV can be differentiated upon clinical presentation (Paper II).

## 2.1 Paper 1

Cover sheet:

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# RESEARCH PAPER COVER SHEET

**Please note that a cover sheet must be completed for each research paper included within a thesis.**

**SECTION A – Student Details**

| | | | |
|---|---|---|---|
| **Student ID Number** | Lsh1800437 | **Title** | |
| **First Name(s)** | Ludmila | | |
| **Surname/Family Name** | Lobkowicz | | |
| **Thesis Title** | Co-circulating Arboviruses in Latin America: Zika Virus, Chikungunya Virus and Dengue Virus | | |
| **Primary Supervisor** | Dr Eizabeth Brickley | | |

**If the Research Paper has previously been published please complete Section B, if not please move to Section C.**

**SECTION B – Paper already published**

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| Where was the work published? | BMJ Global Health | | |
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| For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary) | LL developed the research question. LL, AR and EB designed the study. LL, AR and NSC conducted the literature search, including article titles and abstracts screening, full text paper screening and data extraction. LL took the lead on creating the figures and writing the article. Additionally, all authors supported this work in an advisory capacity and editing the writing of the article and approving its final version. |

**SECTION E**

| | |
|---|---|
| **Student Signature** | [redacted] |
| **Date** | 25.5.20 |

| | |
|---|---|
| **Supervisor Signature** | Elizabeth B. Brickley [redacted] |
| **Date** | 29 May 2020 |

**Paper I title: The frequency and clinical presentation of Zika virus coinfections: a systematic review**

**Authors:** Ludmila Lobkowicz, Anna Ramond, Nuria Sanchez Clemente, Ricardo Arraes de Alencar Ximenes, Demócrito de Barros Miranda-Filho, Ulisses Ramos Montarroyos, Celina Maria Turchi Martelli, Thalia Velho Barreto de Araújo, Elizabeth B Brickley

**Author contribution:**

LL developed the research question. LL, AR and EB designed the study. LL, AR and NSC conducted the literature search, including article titles and abstracts screening, full text paper screening and data extraction. LL took the lead on creating the figures and writing the article. Additionally, all authors supported this work in an advisory capacity and editing the writing of the article and approving its final version.

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BMJ Global Health

# The frequency and clinical presentation of Zika virus coinfections: a systematic review

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## ABSTRACT

**Background** There is limited knowledge on the influence of concurrent coinfections on the clinical presentation of Zika virus (ZIKV) disease.

**Methods** To better understand the types, frequencies and clinical manifestations of ZIKV coinfections, we did a systematic review of four databases (PubMed, Embase, Web of Science, LILACS) without restrictions for studies on ZIKV coinfections confirmed by nucleic acid (quantitative real-time-PCR) testing of ZIKV and coinfecting pathogens. The review aimed to identify cohort, cross-sectional, case series and case report studies that described frequencies and/or clinical signs and symptoms of ZIKV coinfections. Conference abstracts, reviews, commentaries and studies with imprecise pathogen diagnoses and/or no clinical evaluations were excluded.

**Results** The search identified 34 articles from 10 countries, comprising 2 cohort, 10 cross-sectional, 8 case series and 14 case report studies. Coinfections were most frequently reported to have occurred with other arthropod-borne viruses (arboviruses); out of the 213 coinfections described, ZIKV infections co-occurred with chikungunya in 115 cases, with dengue in 68 cases and with both viruses in 19 cases. Other coinfecting agents included human immunodeficiency, Epstein-Barr, human herpes and Mayaro viruses, *Leptospira* spp, *Toxoplasma gondii* and *Schistosoma mansoni*. ZIKV-coinfected cases primarily presented with mild clinical features, typical of ZIKV monoinfection; however, 9% of cases in cohort and cross-sectional studies were reported to experience complications.

**Conclusion** Based on the evidence collated in this review, coinfections do not appear to strongly influence the clinical manifestations of uncomplicated ZIKV infections. Further research is needed to confirm whether risk of severe complications is altered when ZIKV infection co-occurs with other infections.

**PROSPERO registration number** CRD42018111023.

### Key questions

**What is already known?**

- As Zika virus (ZIKV) has been most prevalent in subtropical and tropical regions with high burdens of cocirculating infectious agents, a proportion of ZIKV infections occur simultaneously with infections by one or multiple other pathogens; however, it is uncertain whether coinfections may influence ZIKV-related pathology.

**What are the new findings?**

- This systematic review collated the evidence on ZIKV coinfections as published in 34 studies in 10 countries. ZIKV coinfections were most frequently reported in the context of the arthropod-borne viruses, dengue and chikungunya, but were also described in relation to eight other pathogens.
- While the findings of this review suggest that coinfections do not appear to strongly influence the clinical manifestations of uncomplicated ZIKV infections, this review did identify reports of neurological complications in the context of coinfection.

**What do the new findings imply?**

- The findings of this review highlight a need for coordinated and rapid research efforts during future outbreaks to optimise diagnostic testing strategies for detecting coinfections and determining whether they may exacerbate the risk of severe ZIKV complications, such as Guillain-Barré syndrome and congenital Zika syndrome.

## INTRODUCTION

Zika virus (ZIKV) is an *Aedes* mosquito-borne flavivirus that recently emerged in the Americas.[1] First recognised in Brazil in early 2015, the ZIKV epidemic spread explosively, with autochthonous transmission reported in more than 86 countries and territories by 2018.[1] Given the widespread circulation of this emerging infection of public health concern, it is critical that healthcare practitioners can readily recognise ZIKV disease across the full range of its clinical presentations.

Current evidence indicates that ZIKV infections typically present with no or mild clinical features.[1] A 2018 meta-analysis of 23 studies

by Haby and colleagues estimated a prevalence of asymptomatic ZIKV infections of 62% (95% CI 33% to 87%).[2] For symptomatic ZIKV disease, the WHO describes a mild clinical presentation marked by fever, rash, conjunctivitis, myalgia, arthralgia, malaise and headache.[1] Nevertheless, ZIKV is neurotropic and, in a subset of cases, infections have been associated with severe neurological complications, including the polyneuropathy Guillain-Barré syndrome (GBS) and congenital Zika syndrome (CZS), a constellation of congenital central nervous system malformations resulting from the vertical transmission of ZIKV during pregnancy.[3] It has been estimated that GBS arises in approximately 2 per 10 000 ZIKV infections,[1 4] and the absolute risk of adverse birth outcomes (ie, miscarriage, stillbirth, premature birth and CZS) has been reported to range between 7% and 46% in pregnancies with quantitative real-time PCR (qRT-PCR)-confirmed ZIKV infection.[5–8]

Although the clinical presentation of ZIKV monoinfections has been well characterised, one factor that may influence the clinical spectrum of ZIKV disease is coinfection. Given the high incidence of infectious diseases in the subtropical and tropical areas where ZIKV is prevalent, a proportion of all ZIKV infections occur concurrently with infections by one or multiple pathogens.[9] ZIKV disease in the context of coinfection remains inadequately investigated, and it is uncertain whether specific coinfections may influence the presentation and severity of ZIKV-related signs and symptoms. A 2019 literature review by Vogels and colleagues hypothesised that coinfecting agents have the potential to enhance, inhibit, compete with or have no effect on ZIKV replication and the resulting clinical disease.[10] To advance understanding on this topic, this systematic review aims to quantify how frequently ZIKV coinfections occur among ZIKV-infected populations and to investigate whether the clinical course of ZIKV disease in humans is altered in the context of coinfection.

## METHODS

### Search

Four databases (PubMed, Web of Science, LILACs and EMBASE) were searched for publications up to 19 October 2019 using a comprehensive search strategy (online supplementary appendix 1). Keywords and Medical Subject Headings linked to ZIKV, bacterial, parasitic and other viral infectious diseases were used. The search included English, French, Spanish and Portuguese terms. No date or language restrictions were applied. The systematic review was registered in PROSPERO. All study titles and abstracts were screened based on eligibility criteria, and references of included studies were also screened to identify additional eligible articles.

### Study selection and data extraction

Cohort studies, cross-sectional studies, case series and case reports describing coinfections of ZIKV with one or multiple other pathogens, confirmed by nucleic acid testing (eg, qRT-PCR) for ZIKV, and all coinfecting pathogens were eligible for inclusion in the review. Recovery of live pathogens was also considered to be indicative of acute coinfection. Of note, HIV-positive ZIKV cases with HIV suppression were not included in this review. Two reviewers (AR and LL) simultaneously screened studies for eligibility, and any discrepancies were resolved by a third reviewer (EBB). Conference abstracts, reviews, commentaries and studies without nucleic acid confirmation were excluded. Whereas cohort, cross-sectional and case series studies reporting on numbers of ZIKV coinfections without description of signs and symptoms were included to describe the frequency of ZIKV coinfections, studies with no reporting of signs and symptoms of ZIKV coinfections were otherwise excluded from the review. Data extraction was independently performed by two reviewers (AR, LL). From the full-text articles, information on study author, location, year, data source, age and sex of identified cases was extracted. Additional extracted information included frequencies of ZIKV cases with coinfection, types of coinfection, types of diagnostic testing, reported signs and symptoms, noninfectious comorbidities, and types and frequencies of complications. To investigate the frequency of ZIKV coinfections in cohort, cross-sectional and case series studies, the numbers of coinfections out of the total number of qRT-PCR-confirmed ZIKV cases were calculated for the eligible studies. The study quality assessment was conducted using the Oxford Centre for Evidence-based Medicine (OCEBM) Levels of Evidence, March 2009[11]; see online supplementary appendix 2 for details.

### Patient and public involvement

This research was done without patient or public involvement.

## RESULTS

### Study selection

The search initially identified 12 253 titles, of which 12 050 titles were excluded after screening titles and abstracts and removing duplicates (figure 1). Full-text screening was completed for 203 publications, and, ultimately, 34 articles representing coinfections in 10 countries were included (tables 1–4 and figure 2).

### ZIKV coinfection types

ZIKV infections were most frequently reported to occur concurrently with other arthropod-borne viruses (arboviruses). Out of the 213 coinfections examined, there were 115 ZIKV/chikungunya virus (CHIKV) coinfection cases, 68 ZIKV/dengue virus (DENV) coinfection cases and 19 cases coinfected with all three viruses. Other reported ZIKV coinfections included ZIKV/HIV (n=3), ZIKV/*Leptospira* spp (n=2), ZIKV/CHIKV/HIV/*Toxoplasma gondii* (n=1), ZIKV/CHIKV/*Toxoplasma gondii* (n=1), ZIKV/Epstein-Barr virus (EBV)/human herpes viruses-6 (HHV-6) (n=1), ZIKV/herpes simplex virus-1

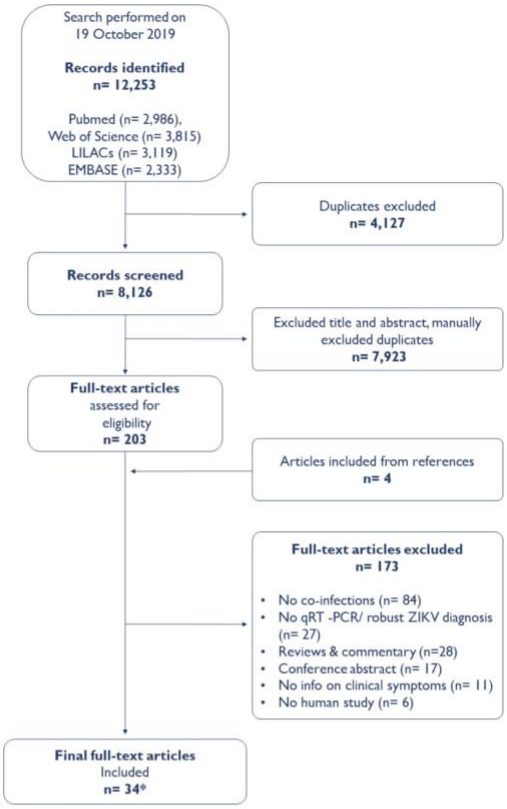


**Figure 1** Study selection.
***Studies reporting on both clinical signs and symptoms and frequency of Zika virus coinfections (n=27); studies reporting only on Zika virus coinfection frequencies (n=7).**
**qRT-PCR, quantitative real-time PCR; ZIKV, Zika virus.**

(HSV-1) (n=1), ZIKV/Mayaro virus (MAYV) (n=1) and ZIKV/*Schistosoma mansoni* (n=1) (figure 3).

### Frequencies of ZIKV coinfections

The frequencies of coinfections among ZIKV-infected populations were reported in 11 studies, including 1 cohort study, 7 cross-sectional studies and 3 case series (table 1, online supplementary table 2). Frequency estimates were reported only for coinfections with CHIKV and DENV and varied geographically and across study populations at risk. Among patients presenting with arbovirus-like symptoms, ZIKV/CHIKV coinfection frequencies were reported to range from 0.3% in a study in Colombia to 54% in a study in Brazil.[5 9 12–14] Similarly, ZIKV/DENV coinfection frequencies in patients with arbovirus-like symptoms were reported to range from 0.03% in a study in Colombia to 47.4% in a study in Brazil.[9 13 15–19] ZIKV/CHIKV/DENV coinfection frequencies ranged from 8% in a study in Nicaragua to 27.6% in a study in Colombia.[9 13]

### Signs and symptoms of coinfections

In total, 27 studies, including 1 cohort study, 5 cross-sectional studies, 7 case series and 14 case report studies, reported the signs and symptoms of ZIKV coinfection across a total of 106 ZIKV-coinfected cases.

### ZIKV/CHIKV coinfections

The clinical presentations of 48 cases with ZIKV/CHIKV coinfection were reported in 1 cohort study, 1 cross-sectional study, 4 case series and 6 case reports (tables 2–4, online supplementary tables 1 and 2).[20–28] Within the cohort, cross-sectional and case series studies, cases were reported to present with the following signs and symptoms consistent with the WHO ZIKV clinical case definition[1]: fever (33%–100%), rash (0%–100%), conjunctivitis (0%–50%), myalgia (67%–100%), arthralgia (0%–67%) and headache (17%–50%) (tables 2 and 3). In addition, gastrointestinal (GI) symptoms were reported in 17% to 100% of cases in three studies (tables 2 and 3).[20 21 23]

Complications were reported among 14.7% (5 cases) of ZIKV/CHIKV-coinfected cases in cohort and cross-sectional studies,[22–27] of whom two adult cases presented with unspecified neurological complications that resulted in death (figure 4).[28] Additionally, two coinfections in pregnancy were associated respectively with anencephaly and an absence of a heartbeat.[28] A non-neurological complication reported was a case that died from multi-organ failure following haemorrhagic manifestations.[23 28] The case series studies described that six out of eight ZIKV/CHIKV-coinfected cases developed complications, which included neurological manifestations, such as GBS in two cases,[22 29] encephalitis in one case,[22] myeloradiculitis in one case,[29] as well as non-neurological complications, such as persistent severe arthralgia in one case.[23] Additionally, four case reports described ZIKV/CHIKV coinfection-associated complications, including GBS in two cases,[24 27] persistent severe arthralgia in one case[26] and sepsis resulting in death in one case.[25]

### ZIKV/DENV coinfections

The clinical features of 42 cases with ZIKV/DENV coinfection were described across four cross-sectional studies, three case series and five case reports (tables 2–4, online supplementary tables 1 and 2).[15 18 19 30–33] Cases with ZIKV/DENV coinfection within the cross-sectional and case series studies were reported to present with the following signs and symptoms consistent with the WHO ZIKV clinical case definition[1]: fever (58%–100%), rash (53%–100%), conjunctivitis (25%–100%), myalgia (75%–100%), arthralgia (50%–100%) and headache (50%–100%) (tables 2 and 3). Other reported clinical features included GI symptoms in 17%–75% of cases and upper respiratory tract (URT) symptoms in 13%–25% of cases (tables 2 and 3).

Complications were reported among none of the ZIKV/DENV-coinfected individuals in cohort and cross-sectional studies (figure 4). However, seven cases with complications were reported in case series, which

**Table 1** ZIKV/CHIKV, ZIKV/DENV and ZIKV/CHIKV/DENV coinfection frequencies among qRT-PCR-confirmed ZIKV infected study population (n=11 studies)

| Author (year) | Country/ Territory | Region | Study year | Study design | Study population* | Coinfecting agent(s) | Coinfection cases (n) | qRT-PCR-confirmed ZIKV infected study population (n) | Frequency (%) | Level of evidence† |
|---|---|---|---|---|---|---|---|---|---|---|
| Mercado-Reyes *et al*[18] (2018) | Colombia | N/A‡ | Oct 2015–Dec 2016 | Cross sectional | Suspected arbovirus infections | CHIKV | 28 | 10118 | 0.3% | 2c |
| Brasil *et al*[5] (2016) | Brazil | South-East | Sept 2015–May 2016 | Cohort | Pregnant women with rash | CHIKV | 3 | 182 | 1.7% | 2b |
| Magalhaes *et al*[12] (2017) | Brazil | North-East | May 2015–May 2016 | Cross sectional | Suspected arbovirus infections | CHIKV | 2 | 26 | 7.7% | 2c |
| Waggoner *et al*[13] (2016) | Nicaragua | N/A | Sept 2015–Apr 2016 | Cross sectional | Suspected arbovirus infections | CHIKV | 16 | 75 | 21.3% | 2c |
| Carrillo- Hernández *et al*[6] (2018) | Colombia | East | Aug 2015–Apr 2016 | Cross sectional | Suspected arbovirus infections | CHIKV | 10 | 29 | 27.6% | 2c |
| Charlys da Costa *et al*[14] (2017) | Brazil | North-East | Mar 2016–May 2016 | Cross sectional | Suspected arbovirus infections with rash | CHIKV | 36 | 66 | 54.0% | 2c |
| Mercado-Reyes *et al*[18] (2018) | Colombia | N/A‡ | Oct 2015–Dec 2016 | Cross sectional | Suspected arbovirus infections | DENV | 3 | 10118 | 0.03% | 2c |
| Chia *et al*[15] (2017) | Singapore | Singapore | Aug 2016–Sept 2016 | Case series | Suspected ZIKV infections | DENV | 4 | 163 | 2.4% | 4 |
| Pessôa *et al*[16] (2016) | Brazil | North-East | May 2015 | Case series | Suspected arbovirus infections | DENV | 1 | 31 | 3.2% | 4 |
| Colombo *et al*[17] (2017) | Brazil | South-East | Jan 2016–Nov 2016 | Cross sectional | Suspected ZIKV infections | DENV | 4 | 100 | 4.0% | 2c |
| Estofolete *et al*[18] (2018) | Brazil | South-East | Jan 2016–Nov 2016 | Case series | Suspected arbovirus infections | DENV | 12 | 151 | 7.9% | 4 |
| Waggoner *et al* (2016)[13] | Nicaragua | N/A | Sept 2015 – Apr 2016 | Cross sectional | Suspected arbovirus infections | DENV | 6 | 75 | 8.0% | 2c |
| Carrillo- Hernández *et al*[6] (2018) | Colombia | East | Aug 2015–Apr 2016 | Cross sectional | Suspected arbovirus infections | DENV | 12 | 29 | 34.4% | 2c |
| Azeredo *et al*[19] (2018) | Brazil | Central-West | Feb 2016–Mar 2016 | Cross sectional | Suspected ZIKV infections | DENV | 18 | 38 | 47.7% | 2c |
| Waggoner *et al*[13] (2016) | Nicaragua | N/A | Sept 2015–Apr 2016 | Cross sectional | Suspected arbovirus infections | CHIKV/ DENV | 6 | 75 | 8.0% | 2c |
| Carrillo-Hernández *et al*[6] (2018) | Colombia | East | Aug 2015–Apr 2016 | Cross sectional | Suspected arbovirus infections | CHIKV/ DENV | 8 | 29 | 27.6% | 2c |

*Online supplementary table 2 gives details of all study populations in cohort studies and case reports.
†All articles were rated according to level of evidence using the Oxford Centre for Evidence-based Medicine's Levels of Evidence, March 2009.[11]
‡Cases originated from the National Surveillance System in Public Health from Colombia. Therefore, cases come from all over Colombia, with the condition of living in a place 2200 m above sea level.
CHIKV, chikungunya virus; DENV, dengue virus; N/A, not available; ZIKV, Zika virus.

**Table 2** Summary of cohort and cross-sectional studies reporting on signs and symptoms of qRT-PCR-confirmed ZIKV coinfections (n=6 studies)

| Author (year) | Location | Study year | Study design | Study population* | % female | Mean age (years) | N, total study population | Coinfecting agent(s) | N, coinfection cases | Other pathogens tested (negative) | Frequency of WHO ZIKV signs and symptoms (%) | | | | | | Frequency of other reported signs and symptoms (%) | | Level of evidence† |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Rash | Fever | Arthralgia | Conjunctivitis | Myalgia | Headache | URT symptoms | GI symptoms | |
| Mercado-Reyes *et al*[7] (2018) | Colombia | 2015/2016 | Cross-sectional study | Suspected arbovirus infections | 74 | 28 | 23 871 | CHIKV | 28 | DENV | NR | NR | NR | NR | NR | NR | NR | NR | 2c |
| Ball *et al*[20] (2018) | Haiti | 2014/2015 | Cohort study | AFI cases | 48 | 7.5 | 252 | CHIKV | 6 | DENV2/ MAYV | 0 | 33% | 67% | NR | 67% | 17% | NR | 17% | 2b |
| Azeredo *et al*[13] (2018) | Brazil | 2016 | Cross-sectional study | Suspected arbovirus infections | NR | 34 | 134 | DENV‡ | 18§ | CHIKV | 53% | 73% | 87% | 60% | 93% | 67% | 13% | 40%¶ | 2c |
| Mercado-Reyes *et al*[21] (2018) | Colombia | 2015/2016 | Cross-sectional study | Suspected arbovirus infections | 74 | 28 | 23 871 | DENV | 3 | CHIKV | NR | NR | NR | NR | NR | NR | NR | NR | 2c |
| Araújo *et al*[18] (2019) | Brazil | 2014/2016 | Cross-sectional study | AFI cases | NR | NR | 9 | DENV | 1 | CHIKV | 100% | NR | 100% | NR | NR | 100% | NR | 100% | 2c |
| Alva-Urcia *et al*[20] (2016) | Peru | 2016 | Cross-sectional study | AFI cases | 63 | NR | 139 | DENV | 1 | NR | NR | 100% | NR | NR | NR | NR | NR | NR | 2c |
| de Souza Costa *et al*[22] (2019) | Brazil | 2015/2016 | Cross-sectional study | AFI cases | 59 | NR | 453 | MAYV | 1 | DENV, CHIKV, YFV, SLEV, ILHV, ROCV, WNV, EEEV, WEEV, VEEV | NR** | NR** | NR** | NR** | NR** | NR** | NR** | NR** | 2c |

URT symptoms: pharyngitis, sore throat, cough, pharyngeal congestion, adenopathy. GI symptoms: nausea, diarrhoea, vomiting, constipation, stomach ache.
*Online supplementary table 2 gives details of all study populations.
†All articles were rated according to level of evidence using the Oxford Centre for Evidence-based Medicine's Levels of Evidence, March 2009.[11]
‡Seventeen cases of DENV-1 and one case of DENV-4.
§Signs and symptoms were only reported for 15 patients.
¶Forty per cent of cases presented with nausea, and 13% of cases with vomiting.
**Case was described to present with typical ZIKV signs for 3 days, but specific details were not reported.
AFI, acute febrile illness; CHIKV, chikungunya virus; DENV, dengue virus; EEV, east equine encephalitis virus; GI, gastrointestinal; ILHV, Ilheus virus; MAYV, Mayaro virus; NR, not reported; ROCV, Rocio virus; SLEV, Saint Louis encephalitis virus; URT, upper respiratory tract; VEEV, Venezuelan equine encephalitis virus; WEEV, west equine encephalitis virus; WNV, West Nile virus; YFV, yellow fever virus; ZIKV, Zika virus.

**Table 3** Summary of case series studies reporting on signs and symptoms of different qRT-PCR-confirmed ZIKV coinfections (n=7 studies)

| Author (year) | Location | Study year | Study design | Study population* | % female | Mean age (years) | N, total study population | Coinfecting agent(s) | N, coinfection cases | Other pathogens tested (negative) | Frequency of WHO ZIKV signs and symptoms (%) | | | | | | Frequency of other reported signs and symptoms (%) | | Level of evidence† |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Rash | Fever | Arthralgia | Conjunctivitis | Myalgia | Headache | URT symptoms | GI symptoms | |
| Acevedo *et al* (2017)[22] | Ecuador | 2016 | Case series | Cases with neurological symptoms‡ | 38 | 42 | 16 | CHIKV | 3 | § | NR | 100% | 33% | NR | NR | 33% | NR | NR | 4 |
| Metha *et al* (2018)[23] | Brazil | 2015/2016 | Case series | Cases with neurological symptoms§ | 50 | 52 | 22 | CHIKV | 2 | DENV | 100% | 50% | 50% | NR | NR | NR | NR | NR | 4 |
| Sardi *et al* (2016)[13] | Brazil | 2015 | Case series | AVI and of qRT-PCR ZIKV+ infections | NR | NR | 15 | CHIKV | 2 | DENV | 50% | 100% | 50% | 50% | 100% | 50% | NR | 50% | 4 |
| Cabral-Castro *et al* (2016)[21] | Brazil | 2015/2016 | Case series | Suspected DENV infections | NR | NR | 30 | CHIKV | 1 | DENV | 100% | 100% | 0 | 0 | NR | NR | NR | 100% | 4 |
| Estololete *et al* (2018)[16] | Brazil | 2016 | Case series | Suspected arbovirus infections | 42 | 46 | 1254 | DENV | 12 | CHIKV | 58% | 58% | 50% | 25% | 83% | 75% | NR | 17% | 4 |
| Chia *et al* (2017)[15] | Singapore | 2016 | Case series | Suspected ZIKV infections | NR | NR | 163 | DENV | 4 | NR | 100% | 100% | 50% | 50% | 75% | 50% | 25% | 75% | 4 |
| Li *et al* (2017)[11] | Singapore | 2016 | Case series | Suspected ZIKV infections | 50 | 11 | 14 | DENV | 1 | CHIKV | 100% | 100% | 100% | 100% | 100% | 100% | 0 | NR | 4 |
| Acevedo *et al* (2017)[22] | Ecuador | 2015/2016 | Case series | Cases with neurological symptoms‡ | 38 | 42 | 16 | CHIKV, DENV | 4 | § | NR | 50% | NR | NR | NR | 25% | 25% | 25% | 4 |
| Acevedo *et al* (2017)[22] | Ecuador | 2016 | Case series | Cases with neurological symptoms‡ | 38 | 42 | 16 | CHIKV, HIV, Toxo | 1 | § | NR | 100% | NR | NR | NR | 100% | NR | NR | 4 |

URT symptoms: pharyngitis, sore throat, cough, pharyngeal congestion, adenopathy. GI symptoms: nausea, diarrhoea, vomiting, constipation, stomach ache.
*Online supplementary table 2 gives details of all study populations.
†All articles were rated according to level of evidence using the Oxford Centre for Evidence-based Medicine's Levels of Evidence, March 2009[11]
‡Associated with suspected arbovirus infection.
§Tested for DENV, gram stain, HSV1/2/6, CMV, EBV, VZ, Toxo, MTB, enterovirus.
AVI, acute viral illness; CHIKV, chikungunya virus; CMV, cytomegalovirus; DENV, dengue virus; GI, gastrointestinal; HSV, herpes simplex virus; MT, Mycobacterium tuberculosis ; NR, not reported; Toxo, Toxoplasma gondii; URT, upper respiratory tract; VZ, varicella zoster.

**Table 4** Summary of case reports reporting on signs and symptoms of qRT-PCR-confirmed ZIKV coinfections (n=21 reports)

| Author (year) | Location | Study year | Sex | Age (years) | Coinfecting agent(s) | Other pathogens tested (negative) | WHO ZIKV signs or symptoms | | | | Other reported signs or symptoms | | | Additional information | Level of evidence* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Rash | Fever | Arthralgia | Conjunctivitis | Myalgia | Headache | URT symptoms | GI symptoms | |
| Brito *et al*[24] (2017) | Brazil | 2016 | M | 74 | CHIKV | DENV, HIV, CMV, HTLV, Schisto, HSV1/2 cysticercosis | NR | † | † | NR | NR | NR | NR | † | **Complications:** Meningoencephalitis associated with Guillain-Barré syndrome (EMG confirmed) | 5 |
| Silva *et al* (2018) | Brazil | 2016 | M | 30 | CHIKV | DENV, bacterial/fungal and MTB infections | NR | † | † | NR | † | NR | NR | NR | **Comorbidities:** Systemic lupus erythematosus **Complications:** Persistent fever for 5 weeks and renal dysfunction. 2 months postinfection severe arthralgia, requiring arthroplasty. 10 months postinfection acute deterioration in renal function, respiratory insufficiency and sepsis. **Outcome:** Death | 5 |
| Cherabuddi *et al*[26] (2016) | Colombia | 2016 | F | 40 | CHIKV | DENV | † | † | † | † | NR | NR | NR | NR | Back pain, retro-ocular pain **Complications:** Persistent severe arthralgia after 2 months **Outcome:** Sequelae | 5 |
| Zambrano *et al*[27] (2016) | Ecuador | 2016 | M | 43 | CHIKV | DENV | † | † | † | † | NR | NR | † | NR | **Outcome:** Full recovery | 5 |
| Zambrano *et al*[27] (2016) | Ecuador | 2016 | F | 43 | CHIKV | NR | ‡ | † | ‡ | † | † | † | NR | NR | **Outcome:** Full recovery | 5 |
| Zambrano *et al*[27] (2016) | Ecuador | 2016 | F | 57 | CHIKV | DENV | NR | † | NR | NR | NR | † | NR | NR | Lumbar back pain **Complications:** Guillain-Barré syndrome (EMG confirmed) **Outcome:** Full recovery | 5 |
| Azeredo *et al*[19] (2016) | Brazil | 2016 | F | NR | DENV | CHIKV | † | † | NR | NR | NR | † | NR | † | Retro-orbital pain **Complications:** Suspected vertical transmission, pregnancy outcome alive newborn with functional plagiocephaly a flat spot on the back or side of newborn's skull **Gestational age at infection:** 9 weeks | 5 |

Continued

**Table 4** Continued

| Author (year) | Location | Study year | Sex | Age (years) | Coinfecting agent(s) | Other pathogens tested (negative) | WHO ZIKV signs or symptoms | | | | | Other reported signs or symptoms | | | Additional information | Level of evidence* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Rash | Fever | Arthralgia | Conjunctivitis | Myalgia | Headache | URT symptoms | GI symptoms | | |
| Azeredo *et al*[19] (2018) | Brazil | 2016 | F | NR | DENV | CHIKV | † | † | NR | † | NR | NR | NR | NR | Retro-orbital pain **Complications:** Suspected vertical transmission to deceased newborn with respiratory insuf-ficiency **Gestational age at infection:** 20 weeks | 5 |
| Dupont-Rouzeyrol *et al*[20] (2015) | New Caledonia | NR | F | 38 | DENV 1 | NR | † | † | † | † | † | † | NR | † | Asthenia, retro-ocular pain **Outcome:** Full recovery | 5 |
| Dupont-Rouzeyrol *et al*[20] (2015) | New Caledonia | NR | M | 14 | DENV 3 | NR | NR | † | † | NR | † | † | NR | NR | Asthenia **Outcome:** Full recovery | 5 |
| Iovine *et al*[33] (2017) | United States§ | 2016 | F | 26 | DENV 2 | CHIKV | † | † | NR | † | NR | NR | † | † | Retro-orbital pain, fatigue, malaise, facial flushing **Outcome:** Full recovery | 5 |
| Villamil-Gomez *et al*[35] (2016) | Colombia | NR | F | 33 | CHIKV, DENV | *Plasmodium* spp | † | NR | † | † | ‡ | † | NR | ‡ | Physical examination revealed cervical lymphadenopathy, bipalpebral oedema, and painful oedema in the lower limbs **Outcome:** Weekly obstetric ultrasounds from 14.6 to 29 weeks of gestation were normal | 5 |
| Gunturiz *et al*[36] (2018) | Colombia | 2016 | F | 18 | CHIKV, Toxo | NR | NR | NR | NR | NR | NR | NR | NR | NR | **Complications:** Suspected vertical transmission of ZIKV infections to the fetus with outcome of fetus diagnosed with CZS at 20 weeks of gestation, termination at 29 weeks of gestation | 5 |
| Villamil-Gomez *et al*[34] (2018) | Colombia | 2015/2016 | M | 28 | HIV | NR | † | † | NR | † | NR | NR | NR | NR | Recently diagnosed with HIV (<1 year), **Lymphocytes T CD4 count (cells/mm³):** 450, **HIV viral load (RNA copies/mL):** 100 **Complication:** Demyelination was found (EMG findings) | 5 |

Continued

**Table 4** Continued

| Author (year) | Location | Study year | Sex | Age (years) | Coinfecting agent(s) | Other pathogens tested (negative) | WHO ZIKV signs or symptoms | | | | | Other reported signs or symptoms | | Additional information | Level of evidence* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Rash | Fever | Arthralgia | Conjunctivitis | Myalgia | Headache | URT symptoms | GI symptoms | |
| Villamil-Gomez *et al*[34] (2018) | Colombia | 2015/2016 | F | 49 | HIV | NR | NR | NR | NR | NR | NR | NR | NR | † | Recently diagnosed with HIV (<1 year)<br>**Lymphocytes T CD4 count (cells/mm³):** 98<br>**HIV viral load (RNA copies/mL):** 1800<br>Hypotension, dysarthria, decreased muscle strength, relaxation of sphincters, areflexia and basal bilateral crackles in the lungs<br>**Complications:** Sepsis | 5 |
| Villamil-Gomez *et al*[34] (2018) | Colombia | 2015/2016 | F | 45 | HIV | NR | † | † | NR | † | NR | NR | NR | † | Recently diagnosed with HIV (1 year ago)<br>**Lymphocytes T CD4 count (cells/mm³):** 380<br>**HIV viral load (RNA copies/mL):** 800<br>**Complication:**<br>Demyelination was found (EMG findings) | 5 |
| Valdespino-Vazquez *et al*[37] (2018) | Mexico | 2016 | F | 22 | EBV, HHV6 | DENV, CHIKV, WNV, VZ, HSV-I/2, HHV7, HHV8,CMV | † | † | NR | NR | NR | † | NR | NR | Infection of pregnant women at 14 weeks of gestation<br>**Complications:**<br>Suspected vertical transmission of ZIKV infections to the foetus with outcome of diagnosed CZS and fetal death at 30 weeks of gestational age, 4 hours after birth | 5 |
| Araujo *et al*[38] (2018) | Brazil | 2016 | M | 26 | HSV-1 | DENV, HSV,VZ, EBV, Toxo, HepC/B, Syphilis spp. | NR | † | NR | NR | NR | † | NR | † | **Complications:**<br>Meningoencephalitis<br>**Outcome:** Full recovery | 5 |
| Biron *et al*[39] (2016) | New Caledonia | NR | M | 19 | *Leptospira* spp | CHIKV, DENV | NR | † | NR | NR | † | NR | † | NR | **Complications:**<br>Haemodynamic condition was unstable, septic shock<br>**Outcome:** Full recovery | 5 |

Continued

**Table 4** Continued

| Author (year) | Location | Study year | Sex | Age (years) | Coinfecting agent(s) | Other pathogens tested (negative) | WHO ZIKV signs or symptoms | | | | | Other reported signs or symptoms | | Additional information | Level of evidence* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Rash | Fever | Arthralgia | Conjunctivitis | Myalgia | Headache | URT symptoms | GI symptoms | | |
| Neaterour *et al* (2017) | Puerto Rico | 2016 | M | 48 | *Leptospira* spp | DENV, CHIKV | NR | † | NR | NR | † | † | NR | † | **Complications:** Severe thrombocytopenia, persistent hypotension (BP=60/40), and onset of haematochezia suggestive of an acute gastrointestinal bleed. Haemodynamic instability and haemorrhagic manifestations. Cardiac arrest. **Outcome:** Death. | 5 |
| Alves *et al*[41] (2017) | Brazil | NR | M | NR, a boy | *Schistosoma mansoni* | NR | † | † | NR | NR | NR | NR | NR | NR | **Complications:** Inflammation of the right testicle and epididymis. The microscopic examination of the testis ruled out the possibility of cancer but confirmed the diagnosis of extensive loss of testicular structure and schistosome egg-induced granulomas. **Outcome:** Full recovery | 5 |

URT symptoms: pharyngitis, sore throat, cough, pharyngeal congestion; adenopathy; GI symptoms: nausea, diarrhoea, vomiting, constipation, stomach ache.
*All articles were rated according to level of evidence using the Oxford Centre for Evidence-based Medicine's Levels of Evidence, March 2009.[1]
†Reported to be present.
‡Reported not to be present.
§Travel associated diagnosed from Haiti.
CHIKV, chikungunya virus; CMV, cytomegalovirus; CZS, congenital Zika syndrome; DENV, dengue virus; EMG, electromyography testing; GI, gastrointestinal; Hep, hepatitis virus; HHV, human herpes virus; HSV, herpes simplex virus; HTLV, human T-lymphotropic virus; MTB, Mycobacterium tuberculosis; NR, not reported; Toxo, Toxoplasma gondii; URT, upper respiratory tract; VZ, varicella zoster; WNV, West Nile virus.

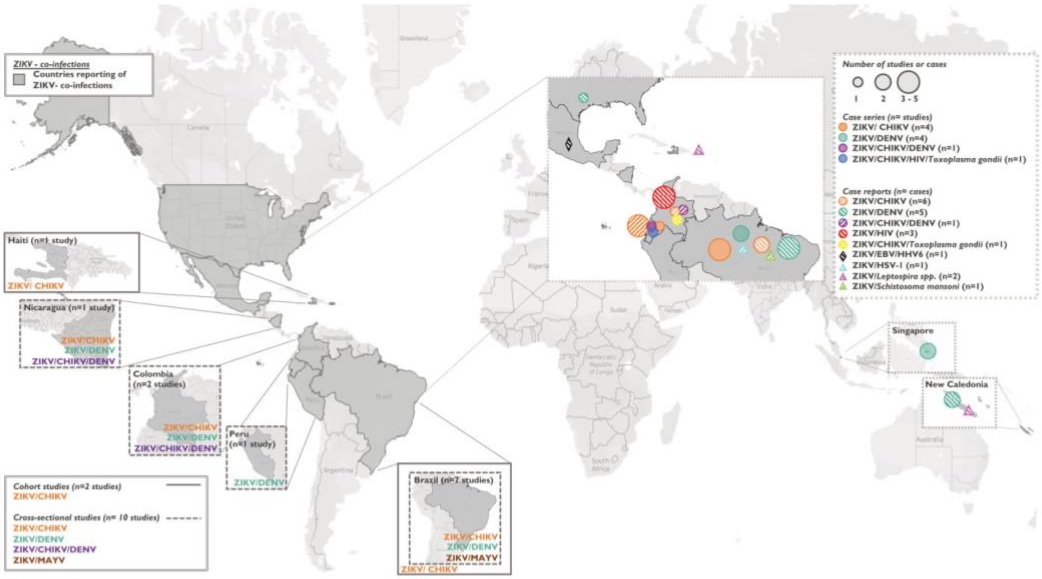


**Figure 2** Studies included in the systematic review: cohort studies (n=2), cross-sectional studies (n=10), case series studies (n=8) and case reports (n=21 reported in 14 case report studies). Two cohort studies on ZIKV/CHIKV coinfections were conducted in Haiti (n=1) and Brazil (n=1). Ten cross-sectional studies were conducted in Brazil (n=6), Colombia (n=2), Nicaragua (n=1) and Peru (n=1). Eight case series were reported from Brazil (n=5), Ecuador (n=1) and Singapore (n=2). Twenty-one case reports were reported from Brazil (n=6), Colombia (n=6), Ecuador (n=3), Mexico (n=1), New Caledonia (n=3), Puerto Rico (n=1) and the USA (n=1). CHIKV, chikungunya virus; DENV, dengue virus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; MAYV, Mayaro virus; ZIKV, Zika virus.

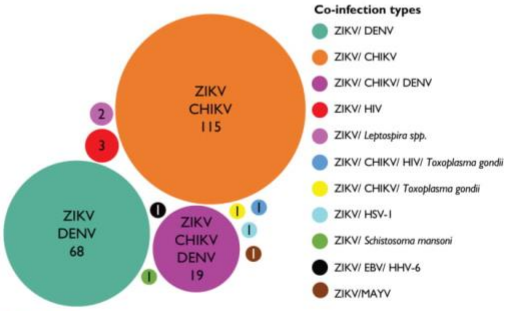


**Figure 3** Zika virus coinfection types identified in this systematic review. Size of circles represents the number of cases reported per coinfection type. In total, 213 coinfection cases were included, ie, ZIKV/CHIKV (n=115), ZIKV/DENV (n=68), ZIKV/CHIKV/DENV (n=19), ZIKV/HIV (n=3), ZIKV/*Leptospira* spp (n=2), ZIKV/HIV/*Toxoplasma gondii* (n=1), ZIKV/CHIKV/*Toxoplasma gondii* (n=1), ZIKV/HSV-1 (n=1), ZIKV/*Schistosoma mansoni* (n=1), ZIKV/EBV/HHV-6 (n=1), ZIKV/MAYV (n=1. CHIKV, chikungunya virus; DENV, dengue virus; EBV, Epstein-Barr virus; HHV, human herpes virus; HSV, herpes simplex virus; MAYV, Mayaro virus; ZIKV, Zika virus.

presented respectively with painful hepatomegaly, liver enlargement, mucosal bleeding, gingival bleeding, significant thrombocytopenia and abrupt platelet decrease.[15 18 19] The only neurological complications resulting from ZIKV/DENV coinfection were reported in two case reports documenting infections in pregnancy, with one case resulting in a newborn with functional plagiocephaly and the other in fetal death (table 3).[19]

### ZIKV/CHIKV/DENV coinfections

The clinical presentation of five cases with ZIKV/CHIKV/DENV coinfection were described in one case series (four cases) and one case report (tables 3 and 4, online supplementary tables 1 and 2).[22 34] Similar to ZIKV/CHIKV and ZIKV/DENV-coinfected cases, ZIKV/CHIKV/DENV-coinfected cases presented with signs and symptoms consistent with the ZIKV WHO clinical case definition.[1] All five cases were reported to have complications (figure 4). The case series reported GBS in two cases, one case of meningitis and one case of encephalitis, which resulted in death. Notably, the study's population was selected to include only clinical patients presenting to hospital with neurological symptoms.[22] The case report documented one case of cervical lymphadenopathy in pregnancy and full recovery.[35]

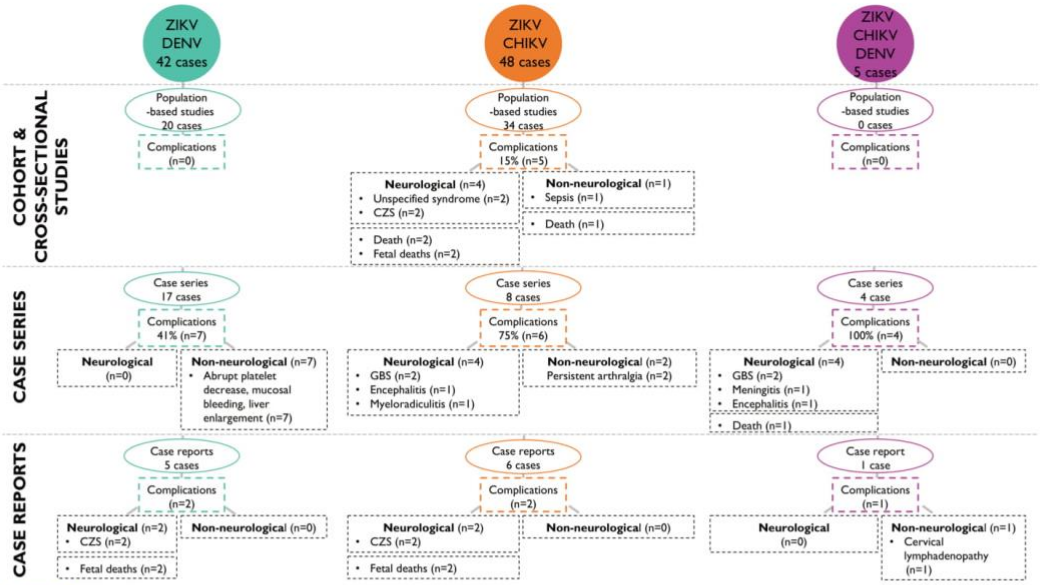


**Figure 4** Complications resulting from Zika virus coinfections with CHIKV and DENV by study design. In cohort and cross-sectional studies, 15% of ZIKV/CHIKV coinfections resulted in complications. In case series, 41% of ZIKV/DENV, 75% of ZIKV/CHIKV and 100% of ZIKV/CHIKV/DENV cases resulted in in complications. In case reports, two ZIKV/DENV, two ZIKV/CHIKV and one ZIKV/CHIKV/DENV coinfections resulted in complications. CHIKV, chikungunya virus; CZS, congenital Zika syndrome; DENV, dengue virus; GBS, Guillain-Barré syndrome; n, number of complications; ZIKV, Zika virus.

### Other ZIKV coinfections

There is limited published evidence on ZIKV coinfections with other pathogens. To date, the clinical signs and symptoms of 10 cases with eight other ZIKV coinfection types have been documented in one cross-sectional study, one case series and seven case reports (tables 2–4, online supplementary tables 1 and 2).[34 36–42]

In addition to presenting with signs and symptoms consistent with the WHO ZIKV clinical case definition, almost all cases of ZIKV coinfections with pathogens other than DENV or CHIKV were reported to experience complications. Neurological complications were reported in two ZIKV/HIV coinfections, one ZIKV/CHIKV/HIV/*Toxoplasma gondii* coinfection and one ZIKV/HSV-1 coinfection. These neurological complications included meningitis, meningoencephalitis and demyelinations confirmed by electromyography.[22 34 38] Further, one ZIKV/HIV-coinfected case developed sepsis, resulting in death.[34] Two ZIKV/*Leptospira* spp-coinfected cases developed haemodynamic instability, one resulting in septic shock,and one in death.[39 40] Additionally, one ZIKV/*Schistosoma mansoni*-coinfected case experienced testicular inflammation with granulomas induced by schistosome eggs.[41]

Coinfections in pregnancy were described in three ZIKV coinfection types: ZIKV/MAYV, ZIKV/CHIKV/*Toxoplasma gondii* and ZIKV/EBV/HHV6 coinfections.[36 37 42] In the latter two, vertical ZIKV transmission was suspected, as both fetuses were diagnosed with CZS. After diagnosis, one pregnancy was terminated at 29 weeks of gestation and one newborn died 4 hours after birth at 30 weeks of gestation due to respiratory distress syndrome.

### Levels of evidence

The levels of evidence for the studies were assessed using the OCEBM Levels of Evidence (1=highest, 5=lowest). Two cohort studies with limited follow-up were graded evidence level 2b.[5 20] Ten cross-sectional studies were graded evidence level 2c.[9 12–14 17 19 28 30 42 43] Eight case series studies were graded evidence level 4.[15 16 18 21–23 29 31] Fourteen case report studies were graded evidence level 5.[19 24–27 32–41] Thus, most of the studies included in the systematic review are evidence level 4 or 5.

## DISCUSSION

This systematic review summarises the existing literature on ZIKV coinfections. Specifically, it describes the estimated frequencies of reported ZIKV coinfections and their clinical spectrum. The search identified 34 studies conducted between 2014 and 2019, which reported 213 cases of ZIKV coinfection with 10 different pathogens. ZIKV coinfections were detected across 10 countries, primarily in Latin America. CHIKV and DENV were the predominantly reported ZIKV coinfecting agents and the

only ZIKV coinfections for which population frequencies were described. ZIKV coinfection frequencies among ZIKV-infected cases varied significantly between location and population type. The vast majority of ZIKV-coinfected cases were reported to present with the signs and symptoms described for uncomplicated ZIKV monoinfections and defined by the WHO.[1] However, complications were reported to arise in 9% of ZIKV-coinfected cases in cohort and cross-sectional studies.

This is the first systematic review to study how frequently individuals with ZIKV infection have a coexisting infection of any kind. The variation in frequencies reported for ZIKV/arbovirus coinfections among the ZIKV-infected individuals reported in this study was likely influenced by differences in study design and the selected study population. Factors, such as study location, season and study period in relation to the ZIKV outbreak, will have additionally influenced ZIKV coinfection frequency estimates. As expected, ZIKV coinfections were relatively more common in studies conducted during concurrent arbovirus outbreaks.[14 44] These differences in study design, timing and location make it difficult to generalise ZIKV coinfection frequency estimates, but provide important knowledge that arbovirus coinfections can occur in up to half of ZIKV-infected cases in certain contexts. Our findings are consistent with a systematic review of CHIKV/DENV coinfections, which found the frequency of CHIKV/DENV coinfections reported in 28 studies ranged from 1% to 36%.[45] The heterogeneity across studies also reflects the difficulty in estimating the background level of ZIKV infections (ie, the denominator for assessing coinfection frequencies), given the diagnostic challenges in identifying acute ZIKV infections.[46]

Overall, the evidence identified in this review suggests that ZIKV coinfections appear to present with a mild clinical presentation similar to that previously described for ZIKV monoinfections. Of note, GI and URT symptoms, which are considered uncharacteristic for ZIKV, were reported to occur not infrequently in ZIKV/DENV, ZIKV/CHIKV and ZIKV/CHIKV/DENV-coinfected cases. While the evidence base from animal model studies of ZIKV coinfection is limited to date, two studies have compared ZIKV infection among rhesus macaque models with and without simian immunodeficiency virus or chimeric simian HIV.[47 48] Whereas coinfected macaques were observed to have lower peak Zika viral loads with a longer clearance time in both investigations, the area under the viral load curves did not appear to differ substantively by coinfection status, potentially suggesting an overall limited impact of coinfection on disease progression but raising questions about the role of lentiviral coinfection in onward transmission.[47 48]

Although the existing reports suggest that coinfections do not appear to markedly alter the clinical presentation of uncomplicated ZIKV disease in humans, the findings from this review highlight a need for additional high quality research investigating whether coinfections may influence complication risks. Based on the limited available evidence, the complications described for ZIKV coinfections appear to be broadly similar to those reported for ZIKV monoinfections.[49] However, 33% of the coinfection-related complications appeared to be atypical for ZIKV monoinfections, but were consistent with complications previously documented for the coinfecting pathogens (eg, bleeding in 10% of ZIKV/DENV cases and persistent arthralgia in 6% of ZIKV/CHIKV cases).[50 51] In addition, among deaths of ZIKV-coinfected cases, three of the nine cases had immune deficiencies and one ZIKV/*Leptospira* spp-coinfected case died from complications established for *Leptospira* spp infections.[40] The remaining five deaths reported from ZIKV coinfections were three fetal deaths, one case following multiorgan failure and one case following encephalitis.[22 28] Additionally, some complications may have been missed, especially those that occurred after the acute infections, as the follow-up period of the individual studies may have not been adequate to detect late-onset complications. Further research (eg, an ongoing cohort study of ZIKV/HIV coinfections in pregnant women[52]) will be valuable for discerning the relative risk of complications of ZIKV coinfection versus monoinfections.

This review had strengths and limitations. ZIKV is an emerging infectious disease of significant public health concern, and this is the first systematic review of the frequency, types and clinical presentation of ZIKV coinfections. The study employed a broad search strategy including search terms for all potential coinfecting pathogens and using multiple languages to identify all available evidence. Most importantly, the review included only qRT-PCR-confirmed ZIKV coinfections, which is the most accurate way to diagnose acute coinfections (ie, due to the very short time window of qRT-PCR testing (<7 days)) and limits misdiagnosis, which is of particular importance with the high cross-reactivity reported from arbovirus serology testing. On the other hand, by focusing on concurrent infections, the current review was unable to appraise the potential impact of recent infections; for example, it has been previously reported that pre-existing immunity to DENV, which shares a common vector and circulates in most of the countries reporting ZIKV coinfection, may influence the clinical presentation of ZIKV infection.[53] The additional limitations of this review mainly stem from the lack of available high-quality evidence on ZIKV coinfections. Notably, the majority of included studies were rated level 4 or 5 according to the OCEBM Levels of Evidence. Only seven studies were rated level 2 or above. Additionally, the reported ZIKV coinfection types may have been influenced by the underlying prevalence of coinfecting pathogens in the population and the applied diagnostic practices (ie, multiplex testing vs testing on clinician's suspicion). The use of specific case definitions in included cross-sectional and case series studies (eg, fever and rash[15]) may have also introduced a selection bias that potentially led to an overrepresentation of specific symptoms associated with ZIKV

coinfection reported for a given study (eg, reporting 100% of cases as presenting with fever and rash).[15] Finally, the studies selected for this systematic review only included symptomatic ZIKV-infected cases, which represent only approximately 40% of all ZIKV cases.[2] It is likely that the actual frequency of ZIKV coinfections may be higher as many cases will be asymptomatic and therefore never seek medical attention. However, the recently implemented multiplex PCR assay, which tests for CHIKV, DENV and ZIKV simultaneously, will likely improve the detection of ZIKV/arbovirus coinfections and facilitate future assessment of the frequency of ZIKV coinfections.[54]

In conclusion, the findings of this review suggest that the cocirculating arboviruses, CHIKV and DENV, are the most common ZIKV coinfection types and may, in specific populations and epidemiological contexts, occur in up to half of ZIKV infections. The evidence collated in this systematic review suggests coinfections do not markedly alter the generally mild clinical presentation of uncomplicated ZIKV disease. However, additional and better quality evidence should be prioritised in future outbreaks to corroborate the estimates of the frequency of ZIKV coinfections and to interrogate the importance of ZIKV coinfections in the development of ZIKV-related complications, especially for ZIKV coinfections with CHIKV and DENV.

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**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

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**Data availability statement** All data relevant to the study are included in the article or uploaded as supplementary information. This systematic review is based on published articles. All abstracted data are provided in the text and supplementary materials.



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## REFERENCES

1 World Health Organization. Zika virus: Factsheet, 2018. Available: https://www.who.int/en/news-room/fact-sheets/detail/zika-virus
2 Haby MM, Pinart M, Elias V, *et al*. Prevalence of asymptomatic Zika virus infection: a systematic review. *Bull World Health Organ* 2018;96:402–13.
3 de Araújo TVB, Rodrigues LC, de Alencar Ximenes RA, *et al*. Association between Zika virus infection and microcephaly in Brazil, January to may, 2016: preliminary report of a case-control study. *Lancet Infect Dis* 2016;16:1356–63.
4 Mier-Y-Teran-Romero L, Delorey MJ, Sejvar JJ, *et al*. Guillain-Barré syndrome risk among individuals infected with Zika virus: a multi-country assessment. *BMC Med* 2018;16:67.
5 Brasil P, Pereira JP, Moreira ME, *et al*. Zika virus infection in pregnant women in Rio de Janeiro. *N Engl J Med* 2016;375:2321–34.
6 Hoen B, Schaub B, Funk AL, *et al*. Pregnancy outcomes after ZIKV infection in French territories in the Americas. *N Engl J Med* 2018;378:985–94.
7 Nogueira ML, Nery Júnior NRR, Estofolete CF, *et al*. Adverse birth outcomes associated with Zika virus exposure during pregnancy in São José do Rio Preto, Brazil. *Clin Microbiol Infect* 2018;24:646–52.
8 Rodriguez-Morales AJ, Cardona-Ospina JA, Ramirez-Jaramillo V, *et al*. Diagnosis and outcomes of pregnant women with Zika virus infection in two municipalities of Risaralda, Colombia: second report of the ZIKERNCOL study. *Travel Med Infect Dis* 2018;25:20–5.
9 Carrillo-Hernández MY, Ruiz-Saenz J, Villamizar LJ, *et al*. 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* 2018;18:61.
10 Vogels CBF, Rückert C, Cavany SM, *et al*. Arbovirus coinfection and co-transmission: a neglected public health concern? *PLoS Biol* 2019;17:e3000130.
11 Phillips B. Oxford Centre for Evidence-based Medicine - Levels of Evidence, 2009. Available: http://www.cebm.net/index.aspx?o=1025
12 Magalhaes T, Braga C, Cordeiro MT, *et al*. Zika virus displacement by a Chikungunya outbreak in Recife, Brazil. *PLoS Negl Trop Dis* 2017;11:e0006055.
13 Waggoner JJ, Gresh L, Vargas MJ, *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.
14 Charlys da Costa A, Thézé J, Komninakis SCV, *et al*. Spread of Chikungunya virus East/Central/South African genotype in northeast Brazil. *Emerg Infect Dis* 2017;23:1742–4.
15 Chia PY, Yew HS, Ho H, *et al*. Clinical features of patients with Zika and dengue virus co-infection in Singapore. *J Infect* 2017;74:611–5.
16 Pessôa R, Patriota JV, Lourdes de Souza Mde, *et al*. Investigation into an outbreak of dengue-like illness in Pernambuco, Brazil, revealed a cocirculation of Zika, Chikungunya, and dengue virus type 1. *Medicine* 2016;95:e3201.
17 Colombo TE, Estofolete CF, Reis AFN, *et al*. Clinical, laboratory and virological data from suspected ZIKV patients in an endemic arbovirus area. *J Clin Virol* 2017;96:20–5.
18 Estofolete CF, Terzian ACB, Colombo TE, *et al*. Co-Infection between Zika and different dengue serotypes during DENV outbreak in Brazil. *J Infect Public Health* 2019;12:178–81.
19 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* 2018;10. doi:10.1371/currents.outbreaks.0bf6aeb4d30824de63c4d5d745b217f5
20 Ball JD, Elbadry MA, Telisma T, *et al*. Clinical and epidemiologic patterns of Chikungunya virus infection and coincident arboviral disease in a school cohort in Haiti, 2014-2015. *Clin Infect Dis* 2019;68:919–26.
21 Cabral-Castro MJ, Cavalcanti MG, Peralta RHS, *et al*. Molecular and serological techniques to detect co-circulation of DENV, ZIKV and CHIKV in suspected dengue-like syndrome patients. *J Clin Virol* 2016;82:108–11.
22 Acevedo N, Waggoner J, Rodriguez M, *et al*. Zika virus, Chikungunya virus, and dengue virus in cerebrospinal fluid from adults with neurological manifestations, Guayaquil, Ecuador. *Front Microbiol* 2017;8:42.
23 Sardi SI, Somasekar S, Naccache SN, *et al*. Coinfections of Zika and Chikungunya viruses in Bahia, Brazil, identified by metagenomic next-generation sequencing. *J Clin Microbiol* 2016;54:2348–53.
24 Brito CAA, Azevedo F, Cordeiro MT, *et al*. Central and peripheral nervous system involvement caused by Zika and Chikungunya coinfection. *PLoS Negl Trop Dis* 2017;11:e0005583.

25 Silva KR, Bica BERG, Pimenta ES, *et al*. Fatal human case of Zika and Chikungunya virus co-infection with prolonged viremia and viruria. *Diseases* 2018;6:53.
26 Cherabuddi K, Iovine NM, Shah K, *et al*. Zika and Chikungunya virus co-infection in a traveller returning from Colombia, 2016: virus isolation and genetic analysis. *JMM Case Rep* 2016;3:e005072–e72.
27 Zambrano H, Waggoner JJ, Almeida C, *et al*. Zika virus and Chikungunya virus coinfections: a series of three cases from a single center in Ecuador. *Am J Trop Med Hyg* 2016;95:894–6.
28 Mercado-Reyes M, Acosta-Reyes J, Navarro-Lechuga E, *et al*. Dengue, Chikungunya and Zika virus coinfection: results of the National surveillance during the Zika epidemic in Colombia. *Epidemiol Infect* 2019;147:e77.
29 Mehta R, Soares CN, Medialdea-Carrera R, *et al*. The spectrum of neurological disease associated with Zika and Chikungunya viruses in adults in Rio de Janeiro, Brazil: a case series. *PLoS Negl Trop Dis* 2018;12:e0006212.
30 Alva-Urcia C, Aguilar-Luis MA, Palomares-Reyes C, *et al*. Emerging and reemerging arboviruses: a new threat in eastern Peru. *PLoS One* 2017;12:e0187897–e97.
31 Li J, Chong CY, Tan NW, *et al*. Characteristics of Zika virus disease in children: clinical, hematological, and virological findings from an outbreak in Singapore. *Clin Infect Dis* 2017;64:1445–8.
32 Dupont-Rouzeyrol M, O'Connor O, Calvez E, *et al*. Co-Infection with Zika and dengue viruses in 2 patients, new Caledonia, 2014. *Emerg Infect Dis* 2015;21:381–2.
33 Iovine NM, Lednicky J, Cherabuddi K, *et al*. Coinfection with Zika and dengue-2 viruses in a traveler returning from Haiti, 2016: clinical presentation and genetic analysis. *Clin Infect Dis* 2017;64:72–5.
34 Villamil-Gómez WE, Sánchez-Herrera Álvaro Rosendo, Hernández-Prado H, *et al*. Zika virus and HIV co-infection in five patients from two areas of Colombia. *J Formos Med Assoc* 2018;117:856–8.
35 Villamil-Gómez WE, Rodríguez-Morales AJ, Uribe-García AM, *et al*. Zika, dengue, and Chikungunya co-infection in a pregnant woman from Colombia. *Int J Infect Dis* 2016;51:135–8.
36 Gunturiz ML, Cortés L, Cuevas EL, *et al*. Congenital cerebral toxoplasmosis, Zika and Chikungunya virus infections: a case report. *Biomedica* 2018;38:144–52.
37 Valdespino-Vázquez MY, Sevilla-Reyes EE, Lira R, *et al*. Congenital Zika syndrome and Extra-Central nervous system detection of Zika virus in a pre-term newborn in Mexico. *Clin Infect Dis* 2019;68:903–12.
38 Araújo PSRde, Silva Júnior MLdeM, Tenório M, *et al*. Co-Infection ZIKV and HSV-1 associated with meningoencephalitis: case report and literature review. *J Infect Public Health* 2019;12:97–100.
39 Biron A, Cazorla C, Amar J, *et al*. Zika virus infection as an unexpected finding in a leptospirosis patient. *JMM Case Rep* 2016;3:e005033–e33.
40 Neaterour P, Rivera A, Galloway RL, *et al*. Fatal *Leptospira* spp./ Zika Virus Coinfection-Puerto Rico, 2016. *Am J Trop Med Hyg* 2017;97:1085–7.
41 Alves LS, Estanislau C, Barreto L, *et al*. Concomitant testicular infection by Zika virus and Schistosoma mansoni in a Brazilian young boy. *Rev Assoc Med Bras* 2017;63:500–3.
42 de Souza Costa MC, Siqueira Maia LM, Costa de Souza V, *et al*. Arbovirus investigation in patients from Mato Grosso during Zika and Chikungunya virus introdution in Brazil, 2015-2016. *Acta Trop* 2019;190:395–402.
43 Beltrán-Silva SL, Chacón-Hernández SS, Moreno-Palacios E, *et al*. Clinical and differential diagnosis: dengue, Chikungunya and Zika. *Revista Médica del Hospital General de México* 2018;81:146–53.
44 Tanabe ELdeL, Tanabe ISB, Santos ECD, *et al*. Report of East-Central South African Chikungunya virus genotype during the 2016 outbreak in the Alagoas state, Brazil. *Rev Inst Med Trop Sao Paulo* 2018;60:e19.
45 Furuya-Kanamori L, Liang S, Milinovich G, *et al*. Co-distribution and co-infection of Chikungunya and dengue viruses. *BMC Infect Dis* 2016;16:84.
46 Ximenes RAA, Miranda-Filho DB, Brickley EB, *et al*. Zika virus infection in pregnancy: establishing a case definition for clinical research on pregnant women with rash in an active transmission setting. *Plos Ntd* 2019.
47 Bidokhti MRM, Dutta D, Madduri LSV, *et al*. SIV/SHIV-Zika co-infection does not alter disease pathogenesis in adult non-pregnant rhesus macaque model. *PLoS Negl Trop Dis* 2018;12:e0006811.
48 Vinton CL, Magaziner SJ, Dowd KA, *et al*. Simian immunodeficiency virus infection of rhesus macaques results in delayed Zika virus clearance. *mBio* 2019;10:e02790–19.
49 Carod-Artal FJ. Neurological complications of Zika virus infection. *Expert Rev Anti Infect Ther* 2018;16:399–410.
50 World Health Organization. Chikungunya virus: Factsheet, 2017. Available: https://wwwwhoint/news-room/fact-sheets/detail/chikungunya
51 World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control : new edition. Available: https://apps.who.int/iris/handle/10665/441882009
52 National Institute of Health. Available: https://clinicaltrials.gov/ct2/show/NCT03263195
53 Rodriguez-Barraquer I, Costa F, Nascimento EJM, *et al*. Impact of preexisting dengue immunity on Zika virus emergence in a dengue endemic region. *Science* 2019;363:607–10.
54 Centers for Disease Control and Prevention. *Diagnostic test for Zika Virus-Trioplex real time RT-PCR assay instructions*. Centre for Disease Control and Prevention, 2016.






Supplementary appendices of Paper I are in the APPENDIX II (p113).

## 2.2 Paper II

Cover sheet:

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# RESEARCH PAPER COVER SHEET

**Please note that a cover sheet must be completed for each research paper included within a thesis.**

**SECTION A – Student Details**

| | | | |
|---|---|---|---|
| **Student ID Number** | Lsh1800437 | **Title** | |
| **First Name(s)** | Ludmila | | |
| **Surname/Family Name** | Lobkowicz | | |
| **Thesis Title** | Co-circulating Arboviruses in Latin America: Zika Virus, Chikungunya Virus and Dengue Virus | | |
| **Primary Supervisor** | Dr Eizabeth Brickley | | |

**If the Research Paper has previously been published please complete Section B, if not please move to Section C.**

**SECTION B – Paper already published**

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**SECTION C – Prepared for publication, but not yet published**

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| Where is the work intended to be published? | The American Journal of Tropical Medicine and Hygiene |
| Please list the paper's authors in the intended authorship order: | Ludmila LOBKOWICZ, Emily L. WEBB, Nuria SANCHEZ CLEMENTE, Jayne WEBSTER, Aisling VAUGHAN, Ulisses Ramos MONTARROYOS, Celina Maria Turchi MARTELLI, Thalia Velho Barreto de ARAÚJO, Wayner Vieira DE SOUZA, Demócrito de Barros MIRANDA- |

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| | FILHO , Ricardo Arraes de Alencar XIMENES, Luciana Caroline Albuquerque BEZERRA, Rafael DHALIA, Ernesto TORRES AZEVEDO MARQUES-JUNIOR, Elizabeth B. BRICKLEY |
| Stage of publication | **Not yet submitted** |

**SECTION D – Multi-authored work**

| | |
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| For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary) | LL developed the research question. LL completed the primary data analysis under the supervision of EW and JW. LL created the figures and wrote the first draft manuscript. Other authors are currently reviewing and providing feedback on the manuscript prior to submission. |

**SECTION E**

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| **Date** | 29 May 2020 |

**Paper 11 title: Co-circulation of Chikungunya virus in a Zika virus outbreak in Recife**

**Author:** Ludmila Lobkowicz, Emily Webb, Nuria Sanchez Clemente, Jayne Webster, Aisling Vaughan, Ulisses Ramos Montarroyos, Celina Maria Turchi Martelli, Thalia Velho Barreto de Araújo, Wayner Vierira de Souza, Demócrito de Barros Miranda-Filho, Ricardo Arraes de Alencar Ximenes, Luciana Caroline Albuquerque, Rafael Dhalia, Ernesto Torres Azevedo Marques-Junior, Elizabeth B Brickley

**Author contribution:**

LL developed the research question. LL completed the data analysis under the supervision of EW. LL created the figures and wrote the first draft manuscript. Other authors are currently reviewing and providing feedback on the manuscript prior to submission.

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**ABSTRACT**

Increased co-circulation of arthropod-borne viruses (arboviruses) has been reported in Latin America and the Caribbean, two of which are Zika virus (ZIKV) and Chikungunya virus (CHIKV). To date, differential diagnosis of these two arboviruses remains complicated, due to their reported overlapping clinical presentations. Our study aimed to describe the co-circulation of ZIKV and CHIKV within a cohort of pregnant women during the outbreak and decline of the ZIKV from 2015 to 2017 in Recife, Pernambuco, Brazil. In addition, we investigated whether the clinical presentations of ZIKV and CHIKV infected cases may be differentiatable. Geographic and temporal CHIKV-ZIKV co-circulation was demonstrated by our findings, based on 213 ZIKV mono-infections, 55 CHIKV mono-infections and 58 sequential ZIKV/CHIKV infections within the cohort. Furthermore, we found that among CHIKV mono-infected cases, certain symptoms, specifically joint pain, joint swelling, fatigue and headache, were more frequently reported than among ZIKV mono-infected cases. Our findings could help healthcare workers to differentiate between CHIKV and ZIKV infections in the event of CHIKV-ZIKV co-circulation in a ZIKV outbreak, in order to guide diagnostic testing and implementation of follow up, and consequently to reduce complications associated with CHIKV and ZIKV infections.

## INTRODUCTION

Arthropod-borne viruses (arboviruses) are a growing threat to public health, increasingly contributing to global disability and mortality. [1-3] Simultaneously, an advancing geographic and temporal co-circulation of arboviruses has been reported worldwide, driven by a combination of factors including urbanization, increased population movement and climate change. [4-6] Growing deforestation and urbanization has led to increased vector-host interaction, while enhanced population movement and changing climate facilitate the spread of viruses (e.g., in infected humans) and vectors (e.g., in containers and ships) to previously unaffected locations. [7,8] Poor sanitation conditions, such as water storage due to limited supply and inadequate sewage disposal, also generate conditions suitable for mosquito proliferation and this is intensified by rising insecticide resistance of mosquitoes. [8,9] In Latin America especially, the co-circulating arboviruses Chikungunya virus (CHIKV), Dengue virus (DENV) and Zika virus (ZIKV) are of increasing public health concern. [10] The three viruses are mainly transmitted by the same vector, *Aedes aegypti*, an urban and peri-urban mosquito, which is present in almost all tropical and subtropical areas. [3,11] Currently over 3 billion people live in regions where *Aedes aegypti* is present and as a result are at risk of arboviral infection. [11]

A major concern regarding the co-circulation of ZIKV, DENV and CHIKV is the accurate discrimination between arboviral infections, as the mild clinical presentation of the three arbovirus has been reported to strongly overlap. [12] To note, CHIKV, DENV and ZIKV infections can be asymptomatic, however whereas DENV and ZIKV infections are thought to be asymptomatic in 60-75% of infections, CHIKV has been described to be asymptomatic in 3-75% cases. [13-16] All three arboviruses present with rash, fever, myalgia, arthralgia, conjunctivitis and headache, however with to date unspecified prevalence of signs and symptoms. [17-19] Additionally, laboratory diagnosis can be challenging, especially in distinguishing between the flaviviruses DENV and ZIKV, for which high cross-reactivity of serological testing has been reported. [20] An accurate differential diagnosis is fundamentally important, as complications differ strongly between ZIKV, DENV and CHIKV infections. ZIKV infection has mainly been associated with the development of neurological complications, such as Guillain-Barré syndrome and adverse birth outcomes such as microcephaly. [19] DENV infection may

lead to the development of dengue haemorrhagic fever, which can lead to dengue shock syndrome, sometimes resulting in death, while CHIKV infections have been associated with neurological complications as well as persistent, disabling severe arthralgia. [17,18] As known complications and long-term sequelae of these infections are becoming increasingly recognized and can lead to severe morbidity, the co-circulation of CHIKV and ZIKV warrants focused investigation.

This study aimed to characterize the geographical and temporal co-circulation of CHIKV and ZIKV within a cohort of pregnant women that presented with rash during the outbreak and decline of the ZIKV from 2015 to 2017 in Recife, Pernambuco, Brazil. [21] Due to the inherent obstacle of serological flavivirus cross-reactivity, DENV infections could not be included in our analyses. In addition, this study investigated whether there were differences in the clinical presentation between ZIKV mono- and CHIKV mono-infections, and between ZIKV-mono-infections and sequentially ZIKV/CHIKV infections in pregnant women, in order to potentially facilitate clinical diagnosis and guide laboratory testing in a situation of ongoing CHIKV-ZIKV co-circulation.

## METHODS

### Ethics

The study was approved by the ethics committee of Fiocruz, Pernambuco, Brazil (Comitê de Ética em Pesquisa do Instituto Aggeu Magalhães (CEP/ CPqAM/Fiocruz)) (1.533.226) and by the ethics committee of the London School of Hygiene and Tropical Medicine (LSHTM Ethics Ref:16412).

### Study design

This was a cross-sectional study, nested within a cohort, which was conducted by the Microcephaly Epidemic Research Group (MERG). [21] The study's population was a cohort of pregnant women who presented with rash (n=694) during the decline of ZIKV in Recife, Pernambuco, Brazil between December 2015 and June 2017. All 694 pregnant within the cohort study were seen at a maximum of three study visits, with some visits taking place after delivery. [21] All 694 pregnant women completed an extensive questionnaire, and blood samples were collected. [21] Detailed information on the design of the cohort study, participants and laboratory procedures has been previously described by Ximenes and colleagues (2019). [21] The median total income per month of people living in the house of a pregnant woman was presented in relation to the Brazilian minimum wage in 2016 (i.e., relative to minimum wage in 2016 880 BRL/Month, the equivalent of about 172 US$/month). [22]

### Laboratory testing

The diagnostic testing of the blood samples was conducted at the Laboratorio de Virologia e Terapia Experimental of the Fundação Oswaldo Cruz (LAVITE-FIOCRUZ, Recife, Pernambuco). [21] Blood samples were tested for ZIKV, by Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) and with primers described by Lanciotti and colleagues, [23] by capture-IgM enzyme-linked immunosorbent assay (ELISA) as described by the United States Centers for Disease Control (CDC, Fort Collins, Colorado, United States of America), [24] and by Plaque Reduction Neutralization Test ($PRNT_{50}$). [21] Additionally, blood samples were tested for CHIKV and DENV by IgM-ELISA (CDC MAC-ELISA). Testing regimes varied across the cohort, as not all ZIKV tests were available or appropriate for blood samples, depending on when the sample was taken. For example qRT-PCR could only be used near the time of acute rash, when viral RNA was still detectable in the

blood sample, and ELISA IgM could only be used approximately 3 days to 2 month after infection, when IgM antibodies have developed in the blood stream. [25] For each woman, a panel of experts, consisting of three virologists, one infectious disease specialist, and one epidemiologist, reviewed all their lab results over time in relation to the dates of rash onset and pregnancy and developed a diagnostic algorithm that defined each individual ZIKV infection in pregnancy. [21] The development of the diagnostic algorithm for ZIKV infection was done blinded to data on later pregnancy outcomes. [21] Recent CHIKV infections and recent DENV infections were identified through IgM.

Due to reported cross-reactivity of DENV IgM tests with acute ZIKV infected serum, true DENV infections could not be differentiated from false positive recent DENV infection results. [20] Therefore, this analysis focused on ZIKV and CHIKV infected pregnant women within the cohort, where flavivirus cross-reactivity does not apply, as CHIKV is an alphavirus. [18] Thus, all pregnant women testing positive for DENV IgM were excluded from the statistical analysis comparing the clinical presentation of ZIKV and CHIKV infections or ZIKV and sequential ZIKV/CHIKV infections. In the cohort we defined "sequential ZIKV/CHIKV infection" as a pregnant woman, with a confirmed ZIKV infection, as described above, and a simultaneaous postive CHIKV IgM test results, confirming recent CHIKV infection (i.e., within 3 to 4 days and up to 2 months after ZIKV infection, defined by the WHO). [26] As infections of TORCH agents (i.e., TORCH - *Toxoplasma gondii*, others (e.g., parvovirus and HIV), rubella virus, cytomegalovirus, herpes simplex virus) in pregnancy are most commonly associated with miscarriage, stillbirth, intrauterine growth restrictions, foetal developmental defects and teratogenicity, the pregnant women were additionally tested for recent infections of TORCH pathogens by ELISA IgM. [27]

**Temporal and geographical investigation of ZIKV and CHIKV co-circulation**

For the investigation of the temporal co-circulation of CHIKV and ZIKV within the pregnant women cohort an epidemiological curve was generated, which depicts pregnant women that tested positive for ZIKV, CHIKV and the total number of pregnant women with rash that were notified within the cohort by epidemiological week (EW) and epidemiological month (EM) over the time period from December 2015 to June 2017. In addition, the effect of seasonality on the number of infections

was assessed graphically, by indicating the epidemiological months of the rainy season in Recife, Pernambuco, Brazil within the graph. [28]

A map was created to visualize the geographical co-circulation of CHIKV and ZIKV within the pregnant women cohort. ArcGIS software (ArcGIS, release 10.5. Redlands, CA: Environmental Systems Research Institute) was used to geo-reference the residence of the pregnant women, providing geographical coordinates (latitude and longitude). The Brazilian Institute of Geography and Statistics (IBGE) website (https://mapas.ibge.gov.br/bases-e-referenciais/bases-cartograficas/malhas-digitais.html) provided a cartographic basis for the city of Recife in the shapefile format in the "geographic" projection system (latitude and longitude) and SIRGAS 2000, which was updated in 2010. [29,30] Using ArcGIS, the residential location of each pregnant woman with positive ZIKV test results was first plotted over a layer of the city map of Recife, on which a layer of pregnant women with positive CHIKV test results was then plotted on top. The map was made at a scale of 1:100,000, which produces an error of approximately 20 m on the real scale (0.2 mm on the map). Therefore, the residence of each pregnant woman is represented as a broad circle of approximately 1250 $m^2$ within a highly urbanized city. The map does not reveal the precise residential location of the pregnant women, therefore ethical concerns regarding identification do not apply.

**Statistical analysis to compare the clinical presentation of ZIKV and CHIKV infections**

In order to assess if there is a difference in the prevalence of signs and symptom between ZIKV and CHIKV mono-infections, first the prevalence of respective signs and symptoms for ZIKV mono- and CHIKV mono-infections was calculated and summarized with the 95% confidence intervals (CI). Crude associations between symptom prevalence of CHIKV and ZIKV mono-infections were assessed using logistic regression (e.g., mathematical formulation to calculate the crude odds ratios of presenting with joint pain when CHIKV infected vs ZIKV infected: logit jointpain i.chikv/zikv), resulting in odds ratios for presenting with a sign or symptom when CHIKV mono-infected vs. ZIKV mono-infected, which were reported with 95% CIs and a p-value from a likelihood ratio test in the logistic model . Logistic regression was also used to adjust for the potential confounder of maternal age, resulting in adjusted odds ratios (e.g., mathematical formulation to calculate odds ratio for presenting

with joint pain when CHIKV infected vs ZIKV infected, adjusted for maternal age: logit jointpain i.chikv/zikv i.maternalage),which were presented with 95% CIs and a p-value from a likelihood ratio test in the logistic model. All statistical analyses described above were also conducted comparing ZIKV mono-infections with sequentially ZIKV/ CHIKV infections.

Finally, predictive modelling was conducted, using logistic regression, to see whether a combination of signs and symptoms is more predictive of being CHIKV mono-infected vs. ZIKV mono-infected than each sign and symptom in isolation, for which odds ratios were presented with a 95% CI and a p-value from a likelihood ratio test in the logistic model.

## RESULTS

### Study population

The study cohort included 694 pregnant women, of which the majority (79%), resided in the Recife metropolitan area (Table 1). The median age of the women was 25.5 years (IQR: 21, 31). The women self-identified with various ethnicities, the most common ethnicity was "pardo" (i.e., mixed race) (65%), followed by "branco" (i.e., white) (23%), followed by "preto" (i.e., black), (10%) and the least frequent ethnicity was Asian (2%). Median schooling years of the women was 10 years (IQR: 8, 11) and median total income per month of people living in the house of a pregnant woman was 1.3 (IQR: 1.0, 2.2) times the Brazilian minimum wage in 2016. Overall, comorbidities during pregnancy were low, apart from gestational hypertension in 20% (n=131) of women and anaemia in 29% (n=179) of women. However, this proportion of anaemia among pregnant women has been reported to be usual for Brazil. [31] Although 10% of women had evidence of recent Herpes simplex virus (HSV) infection (10% were IgM positive), only 2% overall had positive recent serological test results for TORCH agents, including 2% of women were parvovirus IgM, 1% toxoplasmosis IgM and 0.2% cytomegalovirus IgM positive.

### Arbovirus laboratory test results

The laboratory diagnostic results showed that of the 694 women included in the study, 305 (44%) had evidence of an acute ZIKV infection and 145 (21%) had evidence of a recent CHIKV infection (Table 2). [26] The analysis of the temporal and geographical distribution of ZIKV and CHIKV infections was based on data from all 694 pregnant women.

For the analyses of clinical signs and symptoms of ZIKV and CHIKV infections a total of 66 women with DENV IgM positive test results (34 with ZIKV and 32 with CHIKV infections) were excluded, due to reported cross-reactivity of DENV IgM ELISAs with acute ZIKV serum. This left a total of 213 (31%) pregnant women with ZIKV mono-infections, 55 (8%) with CHIKV mono-infections and 58 (8%) sequential ZIKV/CHIKV infections.

**Temporal and geographical investigation of ZIKV and CHIKV co-circulation**

The epidemiological curve shows simultaneous laboratory confirmation of CHIKV and ZIKV infections within the pregnant women cohort in Recife per week between December 2015 and June 2017 (Figure 1). The majority of CHIKV and ZIKV infections occurred between December 2015 and May 2016, with a peak of up to 30 ZIKV infected pregnant women and up to 15 CHIKV infected pregnant women per week. An increase of CHIKV and ZIKV infection notification suggest a strong rise in cases between December 2015 and May 2016 (Figure 1). From June 2016 to August 2016 numbers of CHIKV and ZIKV infections decreased to less than five pregnant women testing positive per week, followed by no new occurring infections between September and November 2016 and isolated CHIKV and ZIKV infections reappearing in pregnant women between December 2016 and June 2017. To note, the rainy season in Recife takes place between the months of March and August each year. [28] The mapping of pregnant women with CHIKV and ZIKV infections on the city of Recife, revealed that pregnant women with CHIKV, ZIKV and sequential ZIKV/CHIKV infections lived in strongly overlapping areas of the city, however without any recognizable pattern differentiating the occurence of CHIKV and ZIKV infections (Figure 2).

**Clinical presentation of ZIKV and CHIKV-infections**

In this cohort, CHIKV mono-infected pregnant women more frequently presented with symptoms compared to ZIKV mono-infected pregnant women within the cohort study (Table 3). This relationship was observed for nearly all symptoms (i.e., joint pain, headache, muscle pain, back ache, fatigue, joint swelling nausea, photophobia, retro-orbital pain and abdominal pain), apart from fever and eye redness which occurred with similar frequency in both groups. In particular, joint pain, joint swelling, fatigue and headache were more common among CHIKV mono-infected pregnant women compared to ZIKV mono-infected pregnant women with an adjusted OR of 2 or more (joint pain: adjusted OR=2.98 (95%CI 1.61-5.28); joint swelling: OR=2.87 (95%CI 1.45-5.65); fatigue OR=2.46 (95%CI 1.26-4.78); headache OR=2.25 (95%CI 1.20-4.20)).

Sequentially ZIKV/CHKV infected pregnant women also presentation more frequently with symptoms compared to ZIKV mono-infected pregnant women within the cohort study (Table 4).

Approximately half of the observed signs and symptoms were more common in sequential ZIKV/CHIKV infected pregnant women than in ZIKV mono-infected pregnant women (i.e., joint pain, headache, muscle pain, back ache, fatigue, and nausea), whereas fever, joint swelling, photophobia, retro-orbital pain, abdominal pain and red eyes were present in similar proportions of pregnant women in both groups. In particular, fatigue, nausea, and joint pain were more common among sequentially ZIKV/CHKV infected pregnant women compared to ZIKV mono-infected pregnant women with an adjusted OR of around 2 or more (fatigue: adjusted OR=2.63 (95%CI 1.38-5.03); nausea: OR=2.54 (95%CI 1.21-5.35); joint pain: OR=1.85 (95%CI 1.01-3.38)).

Predictive modelling of which combination of signs and symptoms would be more indicative for CHIKV mono-infection compared to ZIKV mono-infection, found that when symptoms were combined within the model there was no improvement in model fit, compared to when individual signs and symptoms were included alone in the model, and thus no combinations could be identified to be more indicative for one infection type (Supplementary Table 1). Further investigation revealed that the signs and symptoms were highly correlated (Supplementary Figure 1).

## DISCUSSION

Our study demonstrates geographical and temporal co-circulation of CHIKV and ZIKV within a cohort of pregnant women during the outbreak and decline of the ZIKV between 2015 and 2017 in Recife, Pernambuco, Brazil. In the cohort of pregnant women, those with CHIKV mono-infections presented more frequently with symptoms compared to those with ZIKV mono-infections, with differences most apparent for joint pain, joint swelling, fatigue, retro-orbital pain and headache. Additionally, pregnant women with sequential ZIKV/CHIKV infections presented more frequently with symptoms compared to pregnant women with ZIKV mono-infections, significantly for symptoms such as fatigue, nausea, and joint pain. As CHIKV-ZIKV co-circulation is increasingly being reported, our findings are relevant to facilitate clinical diagnosis and to guide laboratory testing. This is needed to ensure appropriate follow up to prevent and prepare for potential complications associated with CHIKV and ZIKV infection, such as persistant arthralgia, Guillain-Barré syndrome and adverse birth outcomes. Particularly, this study of pregnant women, including their correct clinical diagnosis is highly relevant, as they represent a sub-group particularly at risk of serious complications, especially in the case of ZIKV infection during pregnancy potentially leading to the development of congenital Zika syndrome within the fetus.

Since 2015, when ZIKV was first discovered in Brazil, temporal and geographical CHIKV-ZIKV co-circulation has been frequently described throughout Latin America and the Caribbean (LAC)[32-34]. CHIKV emerged in LAC in 2013, following introduction from Asia to the Caribbean island of Saint-Marteen. [35] By the beginning of 2014 CHIKV had arrived on the LAC mainland and quickly spread throughout the continent. [35] ZIKV meanwhile, was first identified on the LAC mainland in the Northeast of Brazil in May 2015 before spreading to the entire continent. [28] Since 2016, the Brazilian Ministry of Health (BMH) registered ZIKV infections.[36,37] Evidence from the BMH displayed ongoing CHIKV-ZIKV co-circulation in Brazil and specifically in the Northeast of Brazil (i.e., in 2016, 134 ZIKV and 415 CHIKV infections per 100 000 inhabitants registered in the region; with 9 ZIKV and 249 CHIKV infections per 100 000 inhabitants registered in 2017). [38,39] Of note, the State Health Secretariat of Pernambuco and as such Recife's surveillance system made registering ZIKV infection in pregnant

women compulsory since December 2015, as a public health response to the observed microcephaly outbreak. [21] Thus, the sample testing of this cohort study and the epidemic curve resembles the start of the awareness of the potential link between the microcephaly cases and the ZIKV outbreak, hence about 9 months after the actual beginning of the ZIKV outbreak. Therefore, despite the timely acting of MERG, the depicted peak of this study's temporal epidemiological curve most likely does not reflect the true peak of the ZIKV outbreak but should be seen in the light of the new initiation of the surveillance system in December 2015. The peak of the temporal epidemiological curve may be an artefact of notifications, potentially only picking up the decline of the ZIKV cases after the actual peak of the ZIKV outbreak. [40] In line with the temporal findings of our study, the BMH data depicts a decline in ZIKV and CHIKV cases between 2016 and 2017. [38,39] Interestingly, the temporal findings of ZIKV and CHIKV infections in our study did not follow expected seasonal patterns, being high during the dry season of 2015 to 2016 and decreased in the rainy season of 2016. [41] A similar pattern of independence from seasonality has been described in two additional studies; a ZIKV study on a population sample (n=260) in the city of Paulista in the Recife Metropolitan Region, geographically adjacent to our study in 2015/2016 and a DENV time series analysis of surveillance data in the two Brazilian cities, Recife and Goiania between 2001 and 2014. [42,43]

To aid the differential diagnosis of CHIKV and ZIKV infections in a setting with co-circulation, an understanding of the frequency of clinical symptoms of CHIKV and ZIKV in comparison to each other is important. Clinical symptom frequencies for ZIKV and CHIKV infections have commonly been reported in isolation from each other. [44-48] Compared to our study of pregnant women, other studies have described more symptomatic ZIKV mono-infections for both pregnant women and the general population, with higher symptom frequencies especially reported for joint pain, joint swelling, headache, myalgia, and retro-orbital pain. [44,46,48] However, of these studies two reported fever frequencies of around 35%, in contrast to Duffy and colleagues and our study, which report about double the fever frequency among ZIKV mono-infected cases. [44,46,48] The symptom frequencies of CHIKV mono-infections observed in this study are largely supported by the findings of Bagno and colleagues, apart from slightly increased frequencies of arthralgia and myalgia, and only a 40% symptom

frequency of rash compared to the 100% in our study, as by design the women were recruited into our cohort study on the basis of presenting with rash. [49]

To our knowledge only three studies have reported the clinical presentation of CHIKV mono-infections compared to that of ZIKV mono-infections. [34,50,51] One study reported similar clinical presentation of the CHIKV, DENV and ZIKV infections, however the presented frequencies of clinical symptoms caused by ZIKV infections were based on a study population of 31 ZIKV infected cases and on CHIKV and DENV study populations of unreported size. [50] Furthermore, this study did not describe how the comparison between the frequencies of clinical symptoms of ZIKV infections with that of CHIKV and DENV infections was statistically conducted. [44,50] The second study that describes a more severe rash and conjunctival hyperaemia for ZIKV cases compared to DENV and CHIKV cases, also did not describe their sample size or their methods used, to assess signs and symptoms, diagnose cases or statistically compare frequency of signs and symptoms between ZIKV, CHIKV and DENV infections. [51] A study by Waggoner and colleagues on 346 patients with suspected arboviral illness in Nicaragua reported no significant difference in symptom frequencies between 37 ZIKV cases and 103 CHIKV cases, with the exception of rash (91% of ZIKV cases but only in about 56% of CHIKV cases). [34] In contrast to previous reports, our results suggest that the clinical presentation of ZIKV mono-infections significantly differs from that of CHIKV mono-infections and from that of sequential ZIKV/CHIKV infections. Thus, a patient presenting with joint pain, joint swelling, fatigue, retro-orbital pain and headache in addition to rash during an onging ZIKV outbreak, should raise attention in a clinician to test for a potential CHIKV infection. Thus our findings could impact the follow up and clinical management of such patients and thus relieve the public health services economically and capacity-wise (e.g., by eliminating the need of follow up of pregnant CHIKV cases). [52]

Our study has strengths and limitations. Compared to other studies reporting on co-circulation of CHIKV and ZIKV, our study has benefited from a large cohort population with great detail of individual characteristics and of reported signs and symptoms. [34,50,51] To the best of our knowledge, this is also the first study to compare the symptom frequencies of sequential ZIKV/CHIKV infected cases to those of ZIKV mono-infected cases. However, selection bias may have been

introduced into the study as only women presenting with rash were recruited into this study, it is possible our study results on the clinical presentations of CHIKV and ZIKV are not generalisable beyond a ZIKV outbreak setting (i.e., given that rash occurs in around 95% of symptomatic ZIKV infected cases versus only 56% of symptomatic CHIKV infected cases). [34,44,46,48] As a consequence, ZIKV ascertainment in the epidemiological curve and distribution map may have been higher than CHIKV ascertainment. Additionally, information bias may have been introduced during the assessment of signs and symptoms of ZIKV and CHIKV infections, as many symptoms were self-reported. Nevertheless, as pathogen testing was done after assessment of clinical presentation, any misclassification of symptoms was non-differential related to the infection type. Assuming that the misclassification is independent of any other measurement error and non-differential with respect to other variables, the estimates would be biased towards the null, meaning that any true association between signs and symptoms and an infection type may be underestimating. In addition, no formal adjustment was made for multiple testing, but if a more stringent confidence level of 99% had been used, most of the highlighted results would still have been significant. Due to reported issues of cross-reactivity of the DENV IgM assays with acute ZIKV sera, we excluded DENV cases from our analysis of the clinical presentations. [20] This exclusion of DENV cases from our analysis potentially limits the application of our findings on differential diagnosis of ZIKV and CHIKV infections in the event of ZIKV, CHIKV and DENV co-circulation. Furthermore, there is a theoretical possibility of the misclassification of a ZIKV infected pregnant women as a CHIKV IgM diagnosed pregnant women, i.e., if the blood sample was taken in the short timeframe, where ZIKV RNA was no longer present and ZIKV IgM was not yet present in the blood sample. The possibility of misclassification also exists for CHIKV mono-infected women who might have been ZIKV co-infected, as Ximenes and colleagues describe that whereas among pregnant women who tested ZIKV PCR positive, 58% did not become IgM or PRNT positive. [21] Additionally, of the pregnant women who had no evidence of ZIKV infection 42% were not PCR tested. [21] This chance of misclassification however, was counteracted by aiming to take one blood sample at each study visit and conducting diagnostic tests for ZIKV infections using serially collected samples. [21] Additionally, the clinical presentation assessed in this study may be specific to pregnant

women, as immunological alterations have been described in pregnant women potentially leading to altered clinical presentations in pregnancy. [53-55] However, studies have described a very similar clinical presentation of ZIKV and CHIKV infections in pregnancy in comparison to that of the general population, apart from fever which has been described to be a less prevalent symptom of ZIKV infection in pregnant women compared to in the non-pregnant population. [44,56-59]

In conclusion, our findings on the CHIKV-ZIKV co-circulation call for focus on vector control as a potential strategy to reduce overall arbovirus transmission in locations and at time points of high risk arbovirus transmission, and suggest that CHIKV mono-infections are associated with more frequent symptom presentation than ZIKV mono-infections. These differences should be particularly in the forefront of clinicians' thinking when treating patients during ZIKV outbreaks. Despite our findings, we believe that relying on diagnosis upon clinical presentation at this point is insufficient to differentiate between CHIKV, ZIKV, and sequential ZIKV/CHIKV infections. Therefore, especially in pregnant women laboratory testing should be continued to confirm infection type, in order to initiate appropriate and required follow up, and thus reduce CHIKV and ZIKV infections associated complications.

**References**


1 Murray, C. J. *et al.* Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: quantifying the epidemiological transition. *Lancet* **386**, 2145-2191, doi:10.1016/s0140-6736(15)61340-x (2015).

2 Gubler, D. J. & Clark, G. G. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. *Emerging infectious diseases* **1**, 55-57, doi:10.3201/eid0102.952004 (1995).

3 Wilder-Smith, A. *et al.* Epidemic arboviral diseases: priorities for research and public health. *Lancet Infect Dis* **17**, e101-e106, doi:10.1016/s1473-3099(16)30518-7 (2017).

4 Rodriguez-Morales, A. J., Villamil-Gómez, W. E. & Franco-Paredes, C. The arboviral burden of disease caused by co-circulation and co-infection of dengue, chikungunya and Zika in the Americas. *Travel Med Infect Dis* **14**, 177-179, doi:10.1016/j.tmaid.2016.05.004 (2016).

5 Jones, K. E. *et al.* Global trends in emerging infectious diseases. *Nature* **451**, 990-993, doi:10.1038/nature06536 (2008).

6 Wolfe, N. D., Dunavan, C. P. & Diamond, J. Origins of major human infectious diseases. *Nature* **447**, 279-283, doi:10.1038/nature05775 (2007).

7 Weaver, S. C. & Reisen, W. K. Present and future arboviral threats. *Antiviral research* **85**, 328-345, doi:10.1016/j.antiviral.2009.10.008 (2010).

8 Kilpatrick, A. M. & Randolph, S. E. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet (London, England)* **380**, 1946-1955, doi:10.1016/S0140-6736(12)61151-9 (2012).

9 Hemingway, J., Field, L. & Vontas, J. An overview of insecticide resistance. *Science (New York, N.Y.)* **298**, 96-97, doi:10.1126/science.1078052 (2002).

10 Vasconcelos, P. F. & Calisher, C. H. Emergence of Human Arboviral Diseases in the Americas, 2000-2016. *Vector borne and zoonotic diseases (Larchmont, N.Y.)* **16**, 295-301, doi:10.1089/vbz.2016.1952 (2016).

11 Kraemer, M. U. G. *et al.* The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. *eLife* **4**, e08347-e08347, doi:10.7554/eLife.08347 (2015).

12 Musso, D., Cao-Lormeau, V. M. & Gubler, D. J. Zika virus: following the path of dengue and chikungunya? *Lancet (London, England)* **386**, 243-244, doi:10.1016/s0140-6736(15)61273-9 (2015).

13 Schaefer, T. J., Panda, P. K. & Wolford, R. W. in *StatPearls* (StatPearls Publishing LLC., 2019).

14 Haby, M. M., Pinart, M., Elias, V. & Reveiz, L. Prevalence of asymptomatic Zika virus infection: a systematic review. *Bulletin of the World Health Organization* **96**, 402-413d, doi:10.2471/blt.17.201541 (2018).

15 CDC. Chikungunya - Chapter 3 - 2018 Yellow Book | Travelers' Health |. (2018).

16 Dias, J. P. *et al.* Seroprevalence of Chikungunya Virus after Its Emergence in Brazil. *Emerg Infect Dis* **24**, 617-624, doi:10.3201/eid2404.171370 (2018).

17 Organization, W. H. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. *World Health Organization. Geneva.* **1-147.** (2009).

18 Organization, W. H. Chikungunya virus: Fact sheet. https://www.who.int/news-room/fact-sheets/detail/chikungunya (2017).

19 WHO. Zika virus: Factsheet. doi:https://www.who.int/en/news-room/fact-sheets/detail/zika-virus (2018).

20 Wilder-Smith A, V. K., Durbin A, et al. . Zika vaccines and therapeutics: landscape analysis and challenges ahead. . *BMC medicine* **16(1): 84.** (2018).

21 Ximenes, R. A. d. A. *et al.* Zika virus infection in pregnancy: Establishing a case definition for clinical research on pregnant women with rash in an active transmission setting. *PLOS Neglected Tropical Diseases* **13**, e0007763, doi:10.1371/journal.pntd.0007763 (2019).

22 Trading Economics. Minimum wages Brazil. https://tradingeconomics.com/brazil/minimum-wages.

23 Lanciotti, R. S. *et al.* Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* **14**, 1232-1239, doi:10.3201/eid1408.080287 (2008).

24 Prevention., C. f. D. C. a. Zika MAC-ELISA: Instructions for Use. . (2018).

25 Beltrán-Silva, S. L., Chacón-Hernández, S. S., Moreno-Palacios, E. & Pereyra-Molina, J. Á. Clinical and differential diagnosis: Dengue, chikungunya and Zika. *Revista Médica del Hospital General de México* **81**, 146-153, doi:10.1016/j.hgmx.2016.09.011 (2018).

26 WHO. Chikungunya virus: Factsheet. https://www.who.int/news-room/fact-sheets/detail/chikungunya (2017).

27 Adams Waldorf, K. M. & McAdams, R. M. Influence of infection during pregnancy on fetal development. *Reproduction (Cambridge, England)* **146**, R151-162, doi:10.1530/rep-13-0232 (2013).

28 Dantas-Torres F, M. M., Sales K, da Silva FJ, Figueredo LA, Labruna MB. Phenology of Amblyomma sculptum in a degraded area of Atlantic rainforest in north-eastern Brazil. . *Ticks Tick Borne Dis* **10(6): 101263.** (2019).

29 Estatística. IBdGe: SIRGAS Project (Geocentric Reference System for the Americas). . (2016.).

30 Estatística. IBdGe: IBGE _ Cidades _ Infográficos _ Pernambuco _ Recife _ Dados Gerais. . (2019).

31 Machado, Í. E., Malta, D. C., Bacal, N. S. & Rosenfeld, L. G. M. Prevalence of anemia in Brazilian adults and elderly. *Rev Bras Epidemiol* **22Suppl 02**, E190008.SUPL.190002 (2019). <http://europepmc.org/abstract/MED/31596379 https://doi.org/10.1590/1980-549720190008.supl.2>.

32 Carrillo-Hernandez, M. Y., Ruiz-Saenz, J., Villamizar, L. J., Gomez-Rangel, S. Y. & Martinez-Gutierrez, M. Co-circulation and simultaneous co-infection of dengue, chikungunya, and zika viruses in patients with febrile syndrome at the Colombian-Venezuelan border. *BMC infectious diseases* **18**, 61, doi:10.1186/s12879-018-2976-1 (2018).

33 Rico-Mendoza, A., Alexandra, P. R., Chang, A., Encinales, L. & Lynch, R. Co-circulation of dengue, chikungunya, and Zika viruses in Colombia from 2008 to 2018. *Revista panamericana de salud publica = Pan American journal of public health* **43**, e49, doi:10.26633/rpsp.2019.49 (2019).

34 Waggoner, J. J. *et al.* Viremia and Clinical Presentation in Nicaraguan Patients Infected With Zika Virus, Chikungunya Virus, and Dengue Virus. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **63**, 1584-1590, doi:10.1093/cid/ciw589 (2016).

35 Weaver, S. C. & Lecuit, M. Chikungunya virus and the global spread of a mosquito-borne disease. *The New England journal of medicine* **372**, 1231-1239, doi:10.1056/NEJMra1406035 (2015).

36 Brito, C. A. *et al.* Zika in Pernambuco: rewriting the first outbreak. *Rev Soc Bras Med Trop* **49**, 553-558, doi:10.1590/0037-8682-0245-2016 (2016).

37 Ministério da Saúde, B. Monitoramento dos casos de dengue, febre de chikungunya e febre pelo vírus Zika até a Semana Epidemiológica 48, 2015. *Brasília (DF): Ministério da Saúde,* (2015).

38 Saude, S. d. V. e. S.-M. d. Monitoramento dos casos de dengue, febre de chikungunya e febre pelo vírus Zika até a Semana Epidemiológica 50, 2017. (2017).

39 Saúde, S. d. V. e. S. M. d. Monitoramento dos casos de dengue, febre de chikungunya e febre pelo vírus Zika até a Semana Epidemiológica 52, 2016. (2016).

40 Heukelbach, J., Alencar, C. H., Kelvin, A. A., de Oliveira, W. K. & Pamplona de Goes Cavalcanti, L. Zika virus outbreak in Brazil. *Journal of infection in developing countries* **10**, 116-120, doi:10.3855/jidc.8217 (2016).

41 Huber, J. H., Childs, M. L., Caldwell, J. M. & Mordecai, E. A. Seasonal temperature variation influences climate suitability for dengue, chikungunya, and Zika transmission. *PLoS Negl Trop Dis* **12**, e0006451, doi:10.1371/journal.pntd.0006451 (2018).

42 Magalhaes, T. *et al.* Zika virus displacement by a chikungunya outbreak in Recife, Brazil. *PLoS Negl Trop Dis* **11**, e0006055, doi:10.1371/journal.pntd.0006055 (2017).

43 Cortes, F. *et al.* Time series analysis of dengue surveillance data in two Brazilian cities. *Acta tropica* **182**, 190-197, doi:10.1016/j.actatropica.2018.03.006 (2018).

44 Duffy, M. R. *et al.* Zika Virus Outbreak on Yap Island, Federated States of Micronesia. *New England Journal of Medicine* **360**, 2536-2543, doi:10.1056/NEJMoa0805715 (2009).

45 Joob, B. & Wiwanitkit, V. Clinical relevance of Zika symptoms in the context of a Zika Dengue epidemic. *Journal of infection and public health* **13**, 158, doi:10.1016/j.jiph.2019.10.001 (2020).

46 Brasil, P. *et al.* Zika Virus Infection in Pregnant Women in Rio de Janeiro. *The New England journal of medicine* **375**, 2321-2334, doi:10.1056/NEJMoa1602412 (2016).

47 van Genderen, F. T. *et al.* First Chikungunya Outbreak in Suriname; Clinical and Epidemiological Features. *PLoS Negl Trop Dis* **10**, e0004625, doi:10.1371/journal.pntd.0004625 (2016).

48 Guanche Garcell, H. *et al.* Clinical relevance of Zika symptoms in the context of a Zika Dengue epidemic. *Journal of infection and public health*, doi:10.1016/j.jiph.2019.07.006 (2019).

49 Bagno, F. F. *et al.* Undetected Chikungunya virus co-infections in a Brazilian region presenting hyper-endemic circulation of Dengue and Zika. *J Clin Virol* **113**, 27-30, doi:10.1016/j.jcv.2019.02.006 (2019).

50 Ioos, S. *et al.* Current Zika virus epidemiology and recent epidemics. *Med Mal Infect* **44**, 302-307, doi:10.1016/j.medmal.2014.04.008 (2014).

51 Brasil. (Ministério da Saúde, Secretaria de Atenção à Saúde Brasília, DF, 2015).

52 Vouga, M. *et al.* Dengue, Zika and chikungunya during pregnancy: pre- and post-travel advice and clinical management. *Journal of Travel Medicine* **26**, doi:10.1093/jtm/taz077 (2019).

53 Kourtis, A. P., Read, J. S. & Jamieson, D. J. Pregnancy and infection. *The New England journal of medicine* **370**, 2211-2218, doi:10.1056/NEJMra1213566 (2014).

54 Pazos, M., Sperling, R. S., Moran, T. M. & Kraus, T. A. The influence of pregnancy on systemic immunity. *Immunol Res* **54**, 254-261, doi:10.1007/s12026-012-8303-9 (2012).

55 Aghaeepour, N. *et al.* An immune clock of human pregnancy. *Sci Immunol* **2**, doi:10.1126/sciimmunol.aan2946 (2017).

56 Mallet HP, V. A., Musso D. . [Bilan de l'epidemie a virus Zika en polynesie francaise 2013-2014. Bises Bulletin D'informaion Sanitaires, Epidemiologiques Et Statistiques 2015]. . *French. [Accessed August 14, 2016].* (2016).

57 Zika virus infection: global update on epidemiology and potentially associated clinical manifestations. *Wkly Epidemiol Rec* **91**, 73-81 (2016).

58 Ceccaldi PF, L. P., Mandelbrot L. . Infections virales émergentes et grossesse. *Gynecol Obstet Fertil* **35: 339–342.** (2007).

59 Lin, H. Z., Tambyah, P. A., Yong, E. L., Biswas, A. & Chan, S.-Y. A review of Zika virus infections in pregnancy and implications for antenatal care in Singapore. *Singapore Med J* **58**, 171-178, doi:10.11622/smedj.2017026 (2017).

**TABLES**

*Table 1:* **Demographics of the pregnant women cohort in Recife, Pernambuco, Brazil (2015-2017).**

| Characteristics | | No. (% or IQR/ ±SD) |
|---|---|---|
| Residency | Recife metropolitan area | 550 (79%) |
| | Outside Recife metropolitan area | 144 (21%) |
| Age | Median (years) | 26 (21,31) |
| Ethnicity† | Pardo ("mixed race") | 448 (65%) |
| | Branco ("white") | 163 (23%) |
| | Preto ("black") | 69 (10%) |
| | Asian | 12 (2%) |
| Schooling | Median (years) | 10 (8,11) |
| Highest education† | Primary school (incl. equivalency program) | 594 (86%) |
| | Secondary school (incl. equivalency program) | 39 (6%) |
| | Tertiary school incomplete | 38 (6%) |
| | Tertiary school complete | 1 (2%) |
| Inhabitants per household or house | Median | 3 (2, 4) |
| Income (total income of people living in the house per month)† | Median (BRL/month) | 1140 (877, 1915) |
| | relative to minimum wage in 2016 (880.00BRL/Month= 171,97US$/month) | 1.30 (1.0, 2.2) x minimum wage |
| Comorbidities during pregnancy *† | Anaemia | 179 (29%) |
| | Gestational hypertension | 131 (20%) |
| | Diabetes | 19 (3%) |
| | Hypothyroidism | 5 (0.7%) |
| | Chronic kidney disease | 2 (0.2%) |
| | Hypothyroidism | 5 (0.7%) |
| TORCH diagnostics † | Herpes virus IgM | 34 (10%) |
| | Parvovirus IgM | 7 (2%) |
| | *Toxoplasma gondii* IgM | 4 (1%) |
| | CMV IgM | 1 (0.2%) |
| Gestational trimester when notified with rash† | First | 144 (19%) |
| | Second | 226 (38%) |
| | Third | 248 (42%) |
| Previous pregnancy | 0 | 261 (38%) |
| | 1 | 220 (32%) |
| | 2 | 109 (15%) |
| | >=3 | 104 (15%) |
| Previous adverse pregnancy outcomes † | Congenital abnormalities | 18 (5%) |
| | Stillbirth | 17 (5%) |
| | Abortions (spontaneous or induced) | 137 (38%) |
| | Mean no. abortion among women who had abortions ±SD | 1.3 ± 0.72 |

*Reported by pregnant women to have been diagnosed during pregnancy. TORCH- *Toxoplasma gondii*, Other( Syphilis, varicella-zoster, parvovirus, B16), Rubella, Cytomegalovirus (CMV), Herpes virus, Hepatitis B&C, Human immunodeficiency virus (HIV) [60]. †Missing values: Ethnicity (2); Highest education (7); Income (111); Comorbidities: Anaemia (71), Gestational hypertension (23), Diabetes (i.e., before or during pregnancy). (3), Epilepsy (1), Chronic heart disease (2), Chronic kidney disease (4); TORCH diagnostics: *Toxoplasma gondii* IgM (258), Parvovirus IgM (249), CMV IgM (210), Herpes virus IgM (360); Gestational trimester when notified with rash (106); Previous adverse pregnancy outcomes: Congenital abnormalities (303), Stillbirth (367), Abortions (332).

*Table 2:* Arbovirus **diagnostic test results of pregnant women cohort study in Recife, Pernambuco, Brazil**

| Arboviruses tested positive | Testing methods | No. (%) within cohort ($n_{Total}$=694) |
|---|---|---|
| ZIKV | ZIKV (PCR, IgM, PRNT)* | 305 (44%) |
| CHIKV | CHIKV IgM † | 145 (21%) |
| ZIKV mono infection | ZIKV (PCR, IgM, PRNT)* | 213 (31%) |
| CHIKV mono infection | CHIKV IgM | 55 (8%) |
| sequential ZIKV/CHIKV infection | ZIKV (PCR, IgM, PRNT)* and CHIKV IgM | 58 (8%) |

Zika virus (ZIKV), Chikungunya virus (CHIKV), *A panel of virologists, epidemiologists and statisticians agreed upon each individual case of ZIKV positive diagnosis, assessing correlation of qRT-PCR, IgM and PRNT results. [21] † The CHIKV IgM diagnostic tests can detect a recent CHIKV infection (i.e., 3 to 4 days and up to 2 months following symptom onset). [25] The 305 ZIKV infections and 145 CHIKV infections, result from the inclusion of cases with additional recent DENV infections.

*Table 3:* **Prevalence of signs and symptoms of ZIKV mono- vs CHIKV mono-infected pregnant women within the cohort. Crude and adjusted analysis of association of signs and symptoms with ZIKV and CHIKV mono-infection ($n_{Total}$=268).**

| Characteristics * | ZIKV mono-infected | | CHIKV mono-infected | | Crude OR (95% CI)†† | p-value | OR adjusted for maternal age (95% CI)†† | p-value |
|---|---|---|---|---|---|---|---|---|
| | No. of $n_{Total}$ 213 | % (95% CI) † | No. of $n_{Total}$ 55 | % (95% CI)** | | | | |
| Fever | 167 | 78% (72%- 84%) | 44 | 80% (67%- 90%) | 1.08 (0.50- 2.33) | 0.84 | 1.09 (0.51- 2.37) | 0.82 |
| Joint pain (arthralgia) | 64 | 30% (24%- 37%) | 31 | 56% (42%- 70%) | 3.01 (1.63- 5.57) | <0.001 | 2.98 (1.61- 5.28) | 0.001 |
| Headache | 55 | 26% (20%- 32%) | 24 | 44% (30%- 58%) | 2.30 (1.24- 4.29) | 0.0090 | 2.25 (1.20- 4.20) | 0.011 |
| Muscle pain (myalgia) | 54 | 25% (19%- 32%) | 22 | 40% (27%- 54%) | 1.95 (1.04- 3.64) | 0.037 | 1.93 (1.03- 3.62) | 0.041 |
| Back ache | 43 | 20% (15%- 26%) | 18 | 33% (21%- 47%) | 1.97 (1.02- 3.82) | 0.044 | 1.92 (0.99- 3.73) | 0.054 |
| Fatigue | 37 | 17% (13%- 23%) | 19 | 35%, (22%- 49%) | 2.51 (1.30- 4.86) | 0.0060 | 2.46 (1.26- 4.78) | 0.008 |
| Joint swelling (arthritis) | 32 | 15% (10%- 21%) | 19 | 35% (22%- 49%) | 2.97 (1.52- 5.82) | 0.0020 | 2.87 (1.45- 5.65) | 0.0020 |
| Nausea | 24 | 11% (7%– 16%) | 11 | 20% (10%- 33%) | 1.95 (0.89- 4.29) | 0.096 | 2.04 (0.92- 4.52) | 0.078 |
| Photophobia | 19 | 9% (6%-14%) | 7 | 13% (5%- 25%) | 1.54 (0.61- 3.88) | 0.36 | 1.59 (0.63- 4.02) | 0.33 |
| Retro-orbital pain | 18 | 8% (5%-13%) | 11 | 20% (10%- 33%) | 2.80 (1.23- 6.38) | 0.014 | 2.73 (1.19- 6.24) | 0.018 |
| Abdominal pain | 18 | 8% (5%-13%) | 7 | 13% (5%- 25%) | 1.56 (0.62- 4.00) | 0.35 | 1.67 (0.66- 4.28) | 0.28 |
| Eye Redness | 18 | 8% (5%-13%) | 6 | 11% (4%- 22%) | 1.33 (0.50- 3.55) | 0.56 | 1.33 (0.50- 3.55) | 0.57 |

* Cough, sore throat, runny nose, pruritus, secretion of eyes were also reported, however had an overall symptom prevalence with less than 5% in either of the comparison groups, thus no likelihood ratio test was done, due to too low power. Missing values (n) for ZIKV infected: fever (5), muscle pain (6), joint pain (6), joint swelling (6), retro-orbital pain (7), eye redness (7), photophobia(6), headache (5), nausea (6), back ache (5), **Missing values (n) for CHIKV infected: fever (1), muscle pain (1), joint pain (1), joint swelling (1), retro-orbital pain (3), eye redness (2), photophobia(3), headache (2), nausea (1), back ache (2). †† By logistic regression. ZIKV-mono-infection group would be the reference group to calculate OR.

*Table 4:* **Prevalence of signs and symptoms of ZIKV mono- vs sequential ZIKV/CHIKV infection pregnant women cohort. Crude and adjusted analysis of association of signs and symptoms with ZIKV and sequential ZIKV/CHIKV infections ($n_{Total}$=271).**

| Characteristics * | ZIKV mono-infected | | sequential ZIKV/CHIKV infection | | Crude OR (95% CI) †† | p-value | OR adjusted for maternal age (95% CI)†† | p-value |
|---|---|---|---|---|---|---|---|---|
| | No. of $n_{Total}$ 213 | % (95% CI)† | No. of $n_{Total}$ 58 | % (95% CI)** | | | | |
| Fever | 167 | 78% (72%- 84%) | 46 | 79% (67%- 89%) | 1.03 (0.50- 2.16) | 0.95 | 1.10 (0.54- 2.27) | 0.791 |
| Joint pain (arthralgia) | 64 | 30% (24%- 37%) | 26 | 45% (32%- 59%) | 1.87 (1.03- 3.41) | 0.040 | 1.85 (1.01- 3.38) | 0.050 |
| Headache | 55 | 26% (20%- 32%) | 23 | 40% (27%- 53%) | 1.88 (1.02- 3.47) | 0.043 | 1.82 (0.98- 3.37) | 0.060 |
| Muscle pain (myalgia) | 54 | 25% (19%- 32%) | 22 | 38% (26%- 52%) | 1.78 (0.96- 3.30) | 0.067 | 1.76 (0.95- 3.28) | 0.073 |
| Back ache | 43 | 20% (15%- 26%) | 19 | 33% (21%- 46%) | 1.92 (1.01- 3.65) | 0.048 | 1.84 (0.96- 3.52) | 0.066 |
| Fatigue | 37 | 17% (13%- 23%) | 21 | 36% (24%- 50%) | 2.70 (1.42- 5.13) | 0.003 | 2.63 (1.38- 5.03) | 0.003 |
| Joint swelling (arthritis) | 32 | 15% (10%- 21%) | 10 | 17% (9%- 30%) | 1.16 (0.53- 2.53) | 0.71 | 1.14 (0.52- 2.50) | 0.74 |
| Nausea | 24 | 11% (7%– 16%) | 14 | 24% (14%- 37%) | 2.48 (1.19- 5.19) | 0.016 | 2.54 (1.21- 5.35) | 0.014 |
| Photophobia | 19 | 9% (6%-14%) | 7 | 12% (5%-23%) | 1.39 (0.55- 3.47) | 0.49 | 1.42 (0.56- 3.58) | 0.46 |
| Retro-orbital pain | 18 | 8% (5%-13%) | 6 | 10% (4%- 21%) | 1.23 (0.46- 3.26) | 0.68 | 1.21 (0.45- 3.23) | 0.70 |
| Abdominal pain | 18 | 8% (5%-13%) | 6 | 10% (4%- 21%) | 1.24 (0.47- 3.27) | 0.67 | 1.31 (0.49- 3.51) | 0.59 |
| Eye Redness | 18 | 8% (5%-13%) | 7 | 12% (5%-23%) | 1.46 (0.58- 3.49) | 0.42 | 1.42 (0.56- 3.61) | 0.46 |

*Cough, sore throat, runny nose, pruritus, secretion of eyes were also reported, however had an overall symptom prevalence with less than 5% in either of the comparison groups, thus no likelihood ratio test was done, due to too low power. † Missing values (n) for ZIKV infected: fever (5), muscle pain (6), joint pain (6), joint swelling (6), retro-orbital pain (7), eye redness (7), Photophobia(6), Headache (5), Nausea (6), back ache (5), **Missing values (n) for ZIKV and recently CHIKV infected: fever (1), muscle pain (1), joint pain (1), joint swelling (1), retro-orbital pain (1), eye redness (1), photophobia(1), headache (1), nausea (1), back ache (1). †† By logistic regression. ZIKV-mono-infection group would be the reference group to calculate OR.

**FIGURES LEGENDS**

*Figure* 1*:* **Epidemiological curve depicting all notified pregnant women that tested positive for ZIKV (blue) and CHIKV(yellow) and all pregnant women that were notified with rash (black dashes) in the cohort study in Recife, Pernambuco in Brazil (December 2015 - July 2017).** Zika virus (ZIKV), Chikungunya virus (CHIKV), Epidemiological week (EW), Epidemiological month (EM), Epidemiological year (EY), the blue lines above the epidemiological months indicates the months of the rainy season in Recife, Pernambuco, Brazil. [26] The black arrow indicates the beginning of the surveillance system.

Figure 2: **Map of all notified pregnant women that tested positive for ZIKV (blue) (n=108) and CHIKV (yellow) (n=34) and for ZIKV and CHIKV (green) (n=38) in the cohort study in the city of Recife, Pernambuco in Brazil (December 2015 - July 2017). Recife is located in the East of Pernambuco, and Pernambuco is located in the North-East of (small map at the top left).** Zika virus (ZIKV), Chikungunya virus (CHIKV).

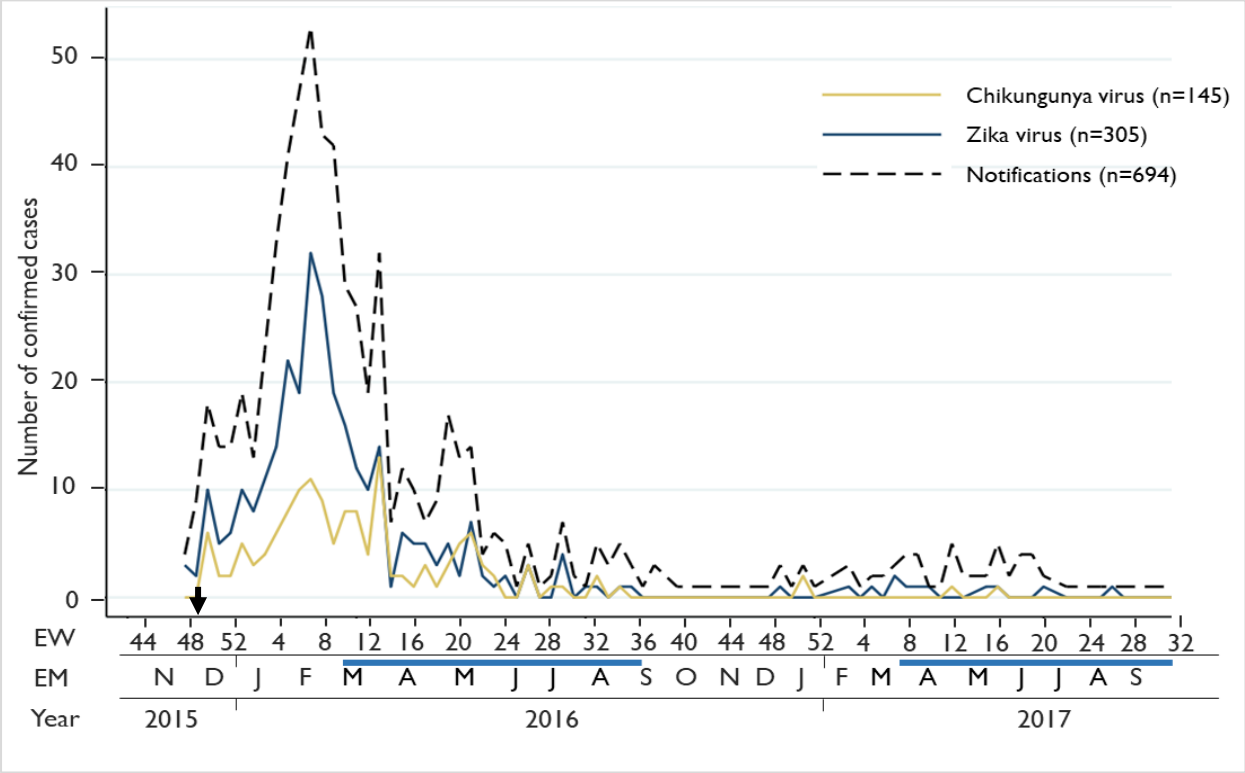


*Figure 5:* **Epidemiological curve depicting all notified pregnant women that tested positive for ZIKV (blue) and CHIKV(yellow) and all pregnant women that were notified with rash (black dashes) in the cohort study in Recife, Pernambuco in Brazil (December 2015 - July 2017).** Zika virus (ZIKV), Chikungunya virus (CHIKV), Epidemiological week (EW), Epidemiological month (EM), Epidemiological year (EY). The blue lines above the epidemiological months indicates the months of the rainy season in Recife, Pernambuco, Brazil. [26] The black arrow indicates the beginning of the surveillance system.

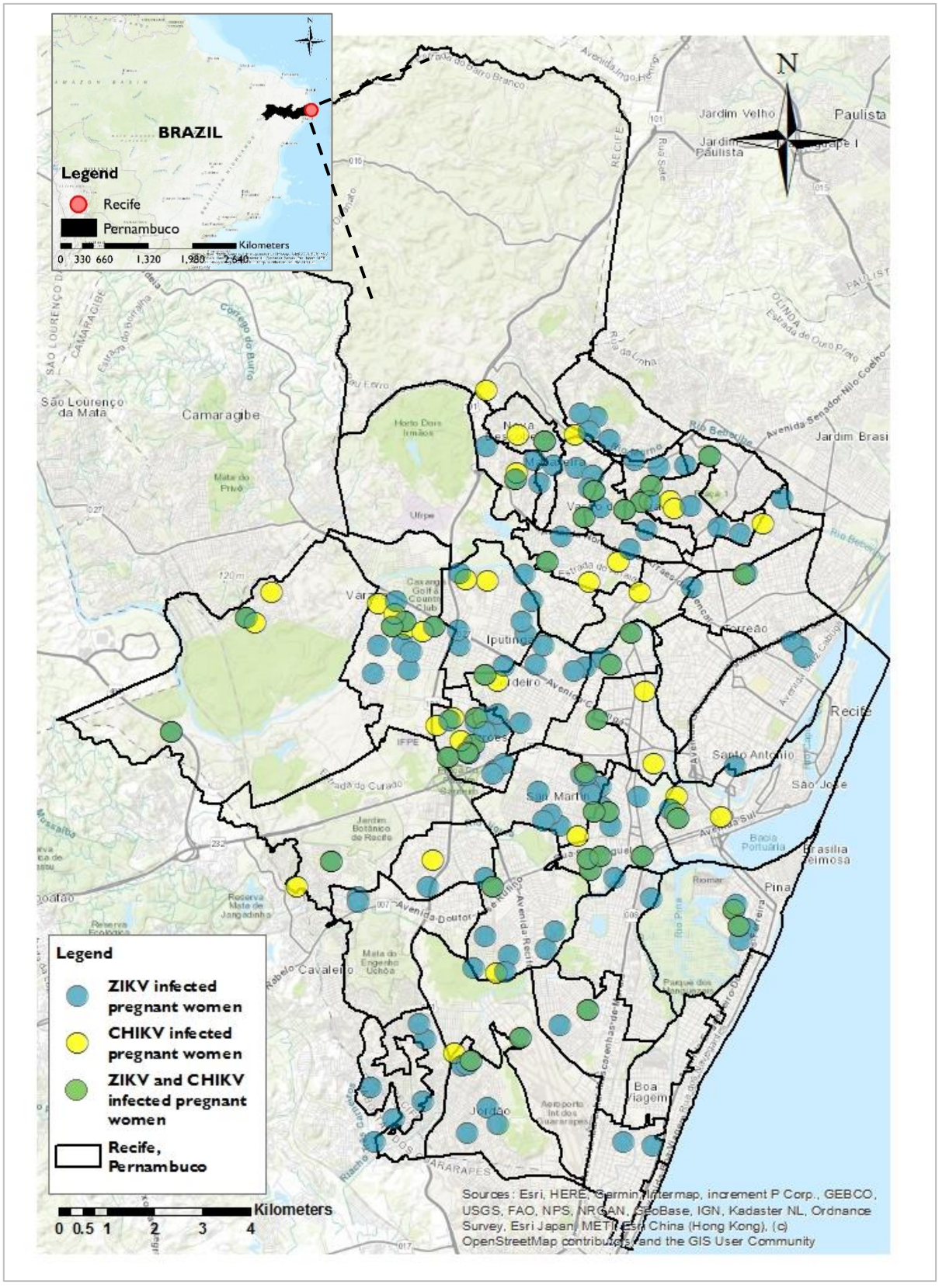


Figure 2: **Map of all notified pregnant women that tested positive for ZIKV (blue) (n=108) and CHIKV (yellow) (n=34) and for ZIKV and CHIKV (green) (n=38) in the cohort study in the city of Recife, Pernambuco in Brazil (December 2015 - July 2017). Recife is located in the East of Pernambuco, and Pernambuco is located in the North-East of (small map at the top left).** Zika virus (ZIKV), Chikungunya virus (CHIKV).

Supplementary appendices of Paper II is in the APPENDIX III (p126)

# 3. CHAPTER III: DISCUSSION

## 3.1 Overview

The overall aim of this study was to investigate co-circulating arboviruses in Latin America in regard to co-infection and differential diagnosis based on clinical presentation, with a focus on the arboviruses of greatest current public health concern in Latin America: CHIKV, DENV and ZIKV. This was done by first systematically reviewing the literature on the frequency and clinical presentation of ZIKV co-infections (paper 1) and by second describing the clinical presentation of ZIKV and CHIKV using data from a pregnant women cohort that presented with rash in Recife, Pernambuco, Brazil between 2015 and 2017 (paper 2).

This chapter contains two main sections. First, I summarized the main findings of each of the two research papers that formed the basis of this research and described their limitations. Second, I discuss the public health implication of this work alongside recommendations for future research.

## 3.2 Summary and interpretation of main findings

The unknown frequency of arbovirus co-infections and their impact on the clinical presentation and the accurate discrimination between arbovirus infections are two central challenges of co-circulating arboviruses in Latin America. The study's systematic review on ZIKV co-infections found that the most commonly reported ZIKV co-infections were with the co-circulating arboviruses CHIKV and DENV, which occurred in specific populations and epidemiological contexts in up to half of ZIKV infections. In contrast to findings of previous studies on arbovirus co-infections, this work suggests co-infections do not distinctly change the generally mild clinical presentation of uncomplicated ZIKV disease, as defined by the WHO[1-3]. However, the available evidence for this systematic review, including the methods used to generate the data, was insufficient to rule out the possibility that the clinical spectrum of ZIKV was influenced by co-infection, in particular as the data lacked a representative sample of ZIKV mono-infections for an appropriate comparison to ZIKV co-infections. Hence, this work was unable to assess whether co-infections lead to an increased incidence of ZIKV-related complications. Nevertheless, to our knowledge, this is the first study to systematically review the frequency and clinical presentation of ZIKV co-infections, and therefore represents important

groundwork for future research.

To facilitate the accurate differentiation between arbovirus infections at the stage of symptom presentation in a setting of arbovirus co-circulation, I believe an understanding of the frequency of arboviruses' clinical symptoms in comparison to each other is important. As mentioned, research to date has mainly reported the respective clinical presentation of CHIKV, ZIKV and DENV infections in isolation from each other, and only three studies have reported a comparison of the clinical presentation of CHIKV mono-infections and ZIKV mono-infections, although with limited sample sizes and limited explanation of quantitative statistical methods used[4-12].

Accordingly, this research set out to address these gaps in knowledge. First, this study's descriptive study described the temporal and geographic co-circulation of CHIKV in a pregnant woman cohort during the outbreak and decline of ZIKV in Recife, Pernambuco, Brazil between 2015 and 2017. Second, in contrast to previous research, the work demonstrated that, in comparison to ZIKV mono-infected pregnant women, CHIKV mono-infected pregnant women presented significantly more frequently with symptoms, such as joint pain, joint swelling, fatigue, retro-orbital pain and headache. Additionally, pregnant women with sequential ZIKV/CHIKV infections presented more frequently with symptoms compared to pregnant women with ZIKV mono-infections, with differences most apparent for fatigue, nausea, and joint pain. Hence, there was substantial overlap between the symptoms identified as more common in CHIKV mono-infected than in ZIKV mono-infected, and those more common in sequential ZIKV/CHIKV infected than in ZIKV mono-infected women, with the exceptions of joint swelling and retro-orbital pain. This overlap suggests that certain clinical features, in particular joint pain and fatigue, are strongly associated with CHIKV both for sequential or mono-infections and should be at the forefront of the clinician's thinking when treating patients in a setting of co-circulating arboviruses. However, the overlap also suggests that if CHIKV is suspected, ZIKV cannot be ruled out. To note, this is the first study to our knowledge that compares symptom frequency of sequentially ZIKV/CHIKV infected, in addition to CHIKV mono-infected, to ZIKV mono-infected cases.

### 3.3 Limitations of main findings

Specific strengths and limitations have been discussed in each paper. In addition, concerning the systematic review, it is likely that not all globally existing ZIKV co-infection types and associated clinical outcomes have yet been reported and that the ZIKV co-infection frequencies overall are underreported. Different causes might have led to the limited data available. When identifying ZIKV co-infections, there might have been economic and practical limitations (i.e., as methods are expensive and need sterile laboratory conditions and working expertise) preventing exhaustive testing and qRT-PCR testing of all potential co-infecting pathogens. In addition, information bias might have been introduced when ZIKV co-infection types were reported, such as the clinician choosing what pathogens to test for (i.e., testing by diagnostic suspicion). Diagnostic suspicion in pathogen testing could be either based on the patient's clinical presentation and geographic pathogen predominance (e.g., patient presented with hemodynamic instability in Puerto Rico, hence the clinician tested for arboviruses and *Leptospira spp*.)[13] or the pregnant state of a female patient, which encourages the testing for any pathogen introducing risk in pregnancy (e.g., testing for ZIKV and *Toxoplasma gondii)*[14]. Thus, diagnostic suspicion inherently risks overlooking other co-infecting pathogens. Furthermore, as the study populations of all studies included in this systematic review only detected symptomatic cases, selection bias might have additionally been introduced, which may have led to the overreporting of the proportion of symptomatic ZIKV co-infections. Additionally, it is likely that the lack of reported asymptomatic ZIKV co-infection might have led to an overall underreporting of ZIKV co-infections. Diagnostic algorithms, such as that of the Brazilian SINAN (i.e., Brazilian Information System for Notifiable Diseases) also lead to a continuing underreporting of arbovirus co-infections, as after one arbovirus diagnosis is confirmed, further testing for co-infecting agents is not encouraged[15]. Publication bias (i.e., systematic differences between published and unpublished evidence) might have additionally impacted the results of the systematic review. For example observational studies without significant findings may be less represented in the appraised literature [16]. Additionally, although useful for describing more severe and rare complications which would otherwise require large sample sizes to detect, case reports are typically biased towards unusual or severe disease presentations. As this study

aimed to investigate the most frequently reported ZIKV co-infection types and their potential to cause more severe clinical outcomes of ZIKV infections, the above mentioned limitations are important to note, however, they do not undermine the value of the findings on ZIKV co-infections with CHIKV and DENV in this systematic review. Overall, an accurate estimate of the prevalence of ZIKV co-infections in different locations would be of public health relevance since it would enable the assessment of the overall magnitude of the potential impact of ZIKV co-infections, especially in the context of ZIKV associated adverse birth and other adverse clinical outcomes.

As described in the introduction, the laboratory diagnosis of arboviruses is complex and presents a challenge for research on arboviruses, especially in a setting of arbovirus co-circulation. This includes the very short time window for accurate arboviral nucleic acid testing, which affects CHIKV, DENV and ZIKV infections as well as the difficult issue of serological cross-reactivity between flaviviruses, which makes the differentiation of DENV and ZIKV by ELISA assays challenging[17-19]. Due to this obstacle, the main limitation of the study's descriptive work was the exclusion of potentially DENV infected pregnant women from the comparison of clinical presentations of arbovirus infections. This could be problematic because although the number of potentially DENV infected women in this pregnant women cohort was low (i.e., about 9% of the total pregnant women cohort), a setting with exclusive co-circulation of ZIKV and CHIKV is unlikely, because DENV transmission is mostly endemic or epidemic in areas of ZIKV and CHIKV transmission[20,21].

Nevertheless, despite this work not having investigated the clinical presentation of CHIKV and ZIKV infections in comparison to DENV infections, previous studies have compared the clinical presentation of CHIKV to DENV infections and ZIKV to DENV infections [22-28]. The most well-powered of these studies was conducted over 3 years (i.e., 2012 to 2015) in Puerto Rico on about 9000 patients with acute febrile illness (AFI), with the aim to distinguish CHIKV from DENV infected and other AFI cases based on robust clinical indicators[23]. In this study 1499 laboratory confirmed CHIKV infected cases were compared to 685 laboratory confirmed DENV infected cases[23]. Interestingly, consistent with our findings on the strong association of joint pain with CHIKV infection, the Puerto Rican study found that swollen joints, joint pain, skin rash, bleeding and irritability were

the most significant positive predictors of a CHIKV infection when comparing CHIKV to DENV infections[23]. In contrast, in the same study a higher proportion of DENV than CHIKV infected cases presented with headache, chills, dizziness, retro-orbital pain, GI symptoms (e.g., nausea, abdominal pain, diarrhoea, vomiting, anorexia), poor circulation, moderate hemoconcentration, severe hemoconcentration and leukopenia[180]. Additionally, another study from Singapore compared the clinical presentation of 34 ZIKV infected cases with that of 87 DENV infected cases[24]. The study identified the presence of conjunctivitis and a normal platelet and monocyte count as positive predictors for ZIKV infection[24]. Despite the low sample size and the study location in Singapore, they argue that this definition has 88% sensitivity and 93% specificity and exceeding accuracy compared to WHO's and CDC's definition, when distinguishing ZIKV from DENV infections[24]. DENV infected cases in the latter study were also described to have presented more frequently with fever and headache than ZIKV infected cases [24]. Thus, evidence suggests that the clinical presentation of DENV infected cases can be distinguished from ZIKV and CHIKV infected cases. However, the main clinical predictors of CHIKV, DENV and ZIKV infections in comparison to each other still have to be investigated.

Furthermore, as resources were limited for qRT-PCR testing of CHIKV and DENV infections in my study I was not able to investigate whether arbovirus co-infections (i.e., ZIKV/CHIKV, ZIKV/DENV, CHIKV/DENV co-infections) presented with a different clinical presentation to arbovirus mono-infection. However, a potential different clinical presentation of arbovirus co-infections should be at the forefront of clinician's minds and should be investigated in future studies.

Finally, the findings of the descriptive study may be limited in generalizability. As mentioned above, this may include factors such as having solely included: a) women presenting with rash (i.e., upon a ZIKV case definition and hence limiting the study's findings to a ZIKV outbreak setting), b) pregnant women (i.e., potentially immunologically altered and exclusively female), c) a population with predominant pre-existing DENV immunity (i.e., potentially influencing the clinical presentation of ZIKV infection[29])[30-32] and d) by only focusing on arbovirus diseases.

### 3.4 Implications and recommendations

Not only did the systematic review contribute to important groundwork on ZIKV co-infections, it also highlighted important knowledge gaps on ZIKV co-infections to be prioritized in future research. Overall, our evidence suggests that DENV and CHIKV have been the most frequently reported ZIKV co-infecting pathogens. Thus, from the perspective of public health relevance, I recommend that future research should be approached in two steps and specifically aimed at ZIKV co-infections with CHIKV and DENV. First, robust estimates of ZIKV/CHIKV, ZIKV/DENV and ZIKV/CHIKV/DENV co-infection frequencies among ZIKV infected should be assessed in cohort studies in different locations in order to estimate the actual worldwide burden of ZIKV co-infections and their potential clinical impact. Second, these cohort studies should serve to precisely define the clinical spectrum and the frequency of complications associated with ZIKV co-infections. Here, in particular neurological complications and adverse birth outcomes should be investigated. These research steps are required to estimate the impact of arbovirus co-infections in order to make public health policy recommendations if needed, such as changing testing algorithms to enhance the detection of arbovirus co-infections and thereby reducing potential associated adverse outcomes.

To note, there are two ongoing prospective cohort studies on ZIKV co-infections. The first cohort study specifically investigates ZIKV/HIV co-infections, with the aim to determine the risk of adverse maternal and child outcomes associated with ZIKV/HIV co-infected pregnant women compared to ZIKV mono-infected pregnant women across clinical sites in the US, Puerto Rico and Brazil (NCT03263195)[33]. The second cohort study is also an ongoing multi-country, prospective cohort study (i.e., in ZIKV endemic regions of Brazil (4 sites), Colombia, Guatemala, Nicaragua, Puerto Rico (2 sites), and Peru), called the Zika in infant and pregnancy (ZIP) study (NCT02856984), that aims to recruit 10 000 pregnant women in order to evaluate the association between ZIKV and pregnancy, neonatal, and infant outcomes[34,35]. One of the ZIP study's secondary objectives is to determine whether co-infections contribute to ZIKV associated adverse birth outcomes[34].

Diagnosing arbovirus infection early in the clinical course is challenging. Nevertheless, it is important in order to guide patient management and administer guidance for timely follow up,

especially for patients during pregnancy and patients who may develop post-acute and/or chronic disease. The early diagnosis of CHIKV mono-infection in pregnancy may relieve women of the psychological stress of expecting adverse birth outcomes associated with maternal ZIKV infections in pregnancy. It will also impact on the follow up and clinical management of such patients and thus assist the public health services financially and capacity-wise[36]. In contrast, identifying ZIKV infections early in pregnancy will facilitate access to the required follow-ups.

Although laboratory diagnosis of arbovirus infections is important for patient care and improving public health, it is often not available or is time-consuming. Thus, in resource poor settings and outbreak scenarios, arbovirus diagnosis often relies on the identification of clinical features, usually consistent with the WHO case definition, which has been described to be of unknown sensitivity and specificity as well as to vary by age groups and timing of specific sign and symptom onset after infection[37-39]. Therefore, our findings on distinguishing CHIKV and ZIKV mono-infections upon clinical presentation in pregnant women may aid healthcare workers to identify CHIKV infected cases in a ZIKV outbreak and potentially improve patient outcomes. Nevertheless, the similar clinical presentation of CHIKV infected and sequentially ZIKV/CHIKV infected pregnant women, displays that ZIKV infection cannot be ruled out a when a CHIKV infection is diagnosed. Furthermore, the early identification of symptomatic patients with acute arbovirus infections may also help to further limit transmission of arboviruses within communities and households. Thus, to improve evidence on distinguishing CHIKV, DENV and ZIKV infections through the identification of clinical features, future cohort studies, such as the above described ZIP study, are needed with greater sample sizes, with study populations including all age groups from both sexes and in areas where all three diseases are common[34]. Such studies should also have the possibility of identifying symptom outcomes together with date post symptom onset. Individual participant data meta-analysis (IPD-MA), such as the ZIKV IPD-MA, could additionally be utilized to compare the data of different cohort studies on ZIKV co-infections[40].

While we could target arbovirus disease individually by developing better diagnostics, treatments and vaccinations, we could also target arbovirus diseases all at once by targeting their

vectors. This approach, however, remains a great challenge throughout the world. To date, our resources of vector control span from self-protection against mosquito bites to community-based mosquito control. Arbovirus disease control programms should strengthen health education to stimulate self-protection against mosquito bites, which currently include using air conditioning, screens, wearing long trousers and sleeves, and using mosquito repellent when outdoors. Nevertheless, there is little evidence on the efficacy of self-protection, and it has been reported to require extensive health education for appropriate use as well as be of limited use due its cost and acceptability in constant use. Control tools such as insecticide treated bed nets, used to prevent the transmission of malaria, are not efficacious for the control of CHIKV, DENV and ZIKV transmission, as *Aedes aegypti* is a day biting vector. Common mosquito control measures include chemical pesticides and mechanical breeding-site reduction (i.e., destruction of breeding sites, such as stored water), but these measures have to date failed to prevent arbovirus transmission in most parts of Latin America.

Alternative mosquito control methods are currently being tested in different locations around the world, including, for example, *Wolbachia* infection in mosquitos and RIDL (Release of Insects with Dominant Lethality)[41]. Mathematical models, developed to predict the effect of *Wolbachia* mosquito strains on DENV transmission, have estimated that these strains could achieve a 66%–75% reduction in the basic reproduction number, $R_0$.[42] Additionally, RIDL have achieved a 95% reduction in local *Aedes* populations in Brazil[41]. As these alternative mosquito control measures are currently still being trialled for safety and ecosystem influences, most affected governments have not yet implemented them.

To note, increasing evidence suggests that tropical arbovirus infections, such as those caused by CHIKV, DENV and ZIKV, mainly occur in poor rural and urban settings and disproportionally affect low-income populations[43-45]. Additionally, arboviral disease outcomes can contribute to poverty by causing long-lasting sequelae and maintaining a cycle of disease, poverty and inequity of access to healthcare[44]. Poverty alone does not promote arboviral outbreaks; however, a community's inability to provide adequate vector control, housing conditions, water supplies, reduced crowding and occupational exposures can perpetuate the spread of these diseases[44]. This highlights that multiple

factors, such as health care education, environmental factors, income inequality, policy making and cultural behaviour, impact on the ongoing co-circulating and transmission of arboviruses and thus future research is needed to understand the correlation of these multiple factors to effectively eliminate arboviral co-circulation and consequently human transmission and infection.

### 3.5 Conclusion

In summary, until mosquito control measures are effective enough to prevent arbovirus transmission, the clinical management of CHIKV, DENV and ZIKV infected patients remains essential. Therefore, the findings presented in this research on ZIKV co-infections and the clinical presentation of ZIKV and CHIKV infected pregnant women contribute to improved patient management and are thus, of great public health relevance. Nevertheless, this work also highlights the need for more research and more understanding and discussion about the co-circulation of arboviruses and the populations living in resource-limited conditions, which are most at risk of arbovirus infections.

### 3.6 References


1 Furuya-Kanamori, L. *et al.* Co-distribution and co-infection of chikungunya and dengue viruses. *BMC infectious diseases* **16**, 84-84, doi:10.1186/s12879-016-1417-2 (2016).

2 Taraphdar, D., Sarkar, A., Mukhopadhyay, B. B. & Chatterjee, S. A comparative study of clinical features between monotypic and dual infection cases with Chikungunya virus and dengue virus in West Bengal, India. *The American journal of tropical medicine and hygiene* **86**, 720-723, doi:10.4269/ajtmh.2012.11-0704 (2012).

3 Chahar, H. S. *et al.* Co-infections with chikungunya virus and dengue virus in Delhi, India. *Emerg Infect Dis* **15**, 1077-1080, doi:10.3201/eid1507.080638 (2009).

4 Duffy, M. R. *et al.* Zika Virus Outbreak on Yap Island, Federated States of Micronesia. *New England Journal of Medicine* **360**, 2536-2543, doi:10.1056/NEJMoa0805715 (2009).

5 Joob, B. & Wiwanitkit, V. Clinical relevance of Zika symptoms in the context of a Zika Dengue epidemic. *Journal of infection and public health* **13**, 158, doi:10.1016/j.jiph.2019.10.001 (2020).

6 Brasil, P. *et al.* Zika Virus Infection in Pregnant Women in Rio de Janeiro. *The New England journal of medicine* **375**, 2321-2334, doi:10.1056/NEJMoa1602412 (2016).

7 van Genderen, F. T. *et al.* First Chikungunya Outbreak in Suriname; Clinical and Epidemiological Features. *PLoS Negl Trop Dis* **10**, e0004625, doi:10.1371/journal.pntd.0004625 (2016).

8 Guanche Garcell, H. *et al.* Clinical relevance of Zika symptoms in the context of a Zika Dengue epidemic. *Journal of infection and public health*, doi:10.1016/j.jiph.2019.07.006 (2019).

9 Ioos, S. *et al.* Current Zika virus epidemiology and recent epidemics. *Med Mal Infect* **44**, 302-307, doi:10.1016/j.medmal.2014.04.008 (2014).

10 Waggoner, J. J. *et al.* Viremia and Clinical Presentation in Nicaraguan Patients Infected With Zika Virus, Chikungunya Virus, and Dengue Virus. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **63**, 1584-1590, doi:10.1093/cid/ciw589 (2016).

11 Brasil. (Ministério da Saúde, Secretaria de Atenção à Saúde Brasília, DF, 2015).

12 Abbink, P., Stephenson, K. E. & Barouch, D. H. Zika virus vaccines. *Nat Rev Microbiol* **16**, 594-600, doi:10.1038/s41579-018-0039-7 (2018).

13 Neaterour, P. *et al.* Fatal Leptospira spp./Zika Virus Coinfection-Puerto Rico, 2016. *The American journal of tropical medicine and hygiene* **97**, 1085-1087, doi:10.4269/ajtmh.17-0250 (2017).

14 Gunturiz, M. L., Cortes, L., Cuevas, E. L., Chaparro, P. & Ospina, M. L. Congenital cerebral toxoplasmosis, Zika and chikungunya virus infections: a case report. *Biomedica : revista del Instituto Nacional de Salud* **38**, 144-152, doi:10.7705/biomedica.v38i0.3652 (2018).

15 SAÚDE, M. R. D. GUIA DE VIGILÂNCIA EM SAÚDE **Volume único, 3a edição , Chapter 7**, pages 422, 423, 430, 434, 435. .

16 Song, F. *et al.* Dissemination and publication of research findings: an updated review of related biases. *Health technology assessment (Winchester, England)* **14**, iii, ix-xi, 1-193, doi:10.3310/hta14080 (2010).

17 Wilder-Smith, A. *et al.* Zika vaccines and therapeutics: landscape analysis and challenges ahead. *BMC Medicine* **16**, 84, doi:10.1186/s12916-018-1067-x (2018).

18 Lanciotti, R. S. *et al.* Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* **14**, 1232-1239, doi:10.3201/eid1408.080287 (2008).

19 World Health Organisation. Chikungunya virus: Fact sheet. . *https://www.who.int/news-room/fact-sheets/detail/chikungunya* (2017).

20 Kosasih, H. *et al.* Evidence for endemic chikungunya virus infections in Bandung, Indonesia. *PLoS neglected tropical diseases* **7**, e2483-e2483, doi:10.1371/journal.pntd.0002483 (2013).

21 Lowe, R. *et al.* The Zika Virus Epidemic in Brazil: From Discovery to Future Implications. *International journal of environmental research and public health* **15**, 96, doi:10.3390/ijerph15010096 (2018).

22 Kularatne, S. A., Gihan, M. C., Weerasinghe, S. C. & Gunasena, S. Concurrent outbreaks of Chikungunya and Dengue fever in Kandy, Sri Lanka, 2006-07: a comparative analysis of clinical

and laboratory features. *Postgraduate medical journal* **85**, 342-346, doi:10.1136/pgmj.2007.066746 (2009).

23 Alvarado, L. I. *et al.* Distinguishing patients with laboratory-confirmed chikungunya from dengue and other acute febrile illnesses, Puerto Rico, 2012-2015. *PLoS neglected tropical diseases* **13**, e0007562, doi:10.1371/journal.pntd.0007562 (2019).

24 Yan, G. *et al.* Distinguishing Zika and Dengue Viruses through Simple Clinical Assessment, Singapore. *Emerging infectious diseases* **24**, 1565-1568, doi:10.3201/eid2408.171883 (2018).

25 Laoprasopwattana, K., Kaewjungwad, L., Jarumanokul, R. & Geater, A. Differential diagnosis of Chikungunya, dengue viral infection and other acute febrile illnesses in children. *The Pediatric infectious disease journal* **31**, 459-463, doi:10.1097/INF.0b013e31824bb06d (2012).

26 Velasco, J. M. *et al.* Chikungunya Virus Infections Among Patients with Dengue-Like Illness at a Tertiary Care Hospital in the Philippines, 2012-2013. *The American journal of tropical medicine and hygiene* **93**, 1318-1324, doi:10.4269/ajtmh.15-0332 (2015).

27 Nkoghe, D. *et al.* No clinical or biological difference between Chikungunya and Dengue Fever during the 2010 Gabonese outbreak. *Infectious disease reports* **4**, e5, doi:10.4081/idr.2012.e5 (2012).

28 Sahadeo, N. *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 neglected tropical diseases* **9**, e0004199, doi:10.1371/journal.pntd.0004199 (2015).

29 Rodriguez-Barraquer, I. *et al.* Impact of preexisting dengue immunity on Zika virus emergence in a dengue endemic region. *Science (New York, N.Y.)* **363**, 607-610, doi:10.1126/science.aav6618 (2019).

30 Kourtis, A. P., Read, J. S. & Jamieson, D. J. Pregnancy and infection. *The New England journal of medicine* **370**, 2211-2218, doi:10.1056/NEJMra1213566 (2014).

31 Pazos, M., Sperling, R. S., Moran, T. M. & Kraus, T. A. The influence of pregnancy on systemic immunity. *Immunol Res* **54**, 254-261, doi:10.1007/s12026-012-8303-9 (2012).

32 Aghaeepour, N. *et al.* An immune clock of human pregnancy. *Sci Immunol* **2**, doi:10.1126/sciimmunol.aan2946 (2017).

33 ClinicalTrials.gov. Prospective Cohort Study of HIV and Zika in Infants and Pregnancy (HIV ZIP)(NCT03263195).

34 Lebov, J. F. *et al.* International prospective observational cohort study of Zika in infants and pregnancy (ZIP study): study protocol. *BMC pregnancy and childbirth* **19**, 282, doi:10.1186/s12884-019-2430-4 (2019).

35 ClinicalTrails.gov. Zika in Infants and Pregnancy (ZIP) (NCT02856984).

36 Vouga, M. *et al.* Dengue, Zika and chikungunya during pregnancy: pre- and post-travel advice and clinical management. *Journal of Travel Medicine* **26**, doi:10.1093/jtm/taz077 (2019).

37 Chikungunya: case definitions for acute, atypical and chronic cases. Conclusions of an expert consultation, Managua, Nicaragua, 20-21 May 2015. *Releve epidemiologique hebdomadaire* **90**, 410-414 (2015).

38 Braga, J. U. *et al.* Accuracy of Zika virus disease case definition during simultaneous Dengue and Chikungunya epidemics. *PLoS One* **12**, e0179725, doi:10.1371/journal.pone.0179725 (2017).

39 van Keulen, V., Huibers, M., Manshande, M., van Hensbroek, M. B. & van Rooij, L. Chikungunya Virus Infections Among Infants-Who Classification Not Applicable. *The Pediatric infectious disease journal* **37**, e83-e86, doi:10.1097/inf.0000000000001826 (2018).

40 Wilder-Smith, A. *et al.* Understanding the relation between Zika virus infection during pregnancy and adverse fetal, infant and child outcomes: a protocol for a systematic review and individual participant data meta-analysis of longitudinal studies of pregnant women and their infants and children. *BMJ open* **9**, e026092, doi:10.1136/bmjopen-2018-026092 (2019).

41 Alphey, L. *et al.* Genetic control of Aedes mosquitoes. *Pathogens and global health* **107**, 170-179, doi:10.1179/2047773213y.0000000095 (2013).

42 Ferguson, N. M. *et al.* Modeling the impact on virus transmission of Wolbachia-mediated blocking of dengue virus infection of Aedes aegypti. *Science translational medicine* **7**, 279ra237, doi:10.1126/scitranslmed.3010370 (2015).

43 Kumar, C. J. *et al.* The socioeconomic impact of the chikungunya viral epidemic in India. *Open Med* **1**, e150-e152 (2007).

44 LaBeaud, A. D. Why Arboviruses Can Be Neglected Tropical Diseases. *PLoS neglected tropical diseases* **2**, e247, doi:10.1371/journal.pntd.0000247 (2008).

45 Campos, M. C. *et al.* Zika might not be acting alone: Using an ecological study approach to investigate potential co-acting risk factors for an unusual pattern of microcephaly in Brazil. *PLoS One* **13**, e0201452, doi:10.1371/journal.pone.0201452 (2018).

# APPENDICES

## *APPENDIX I ETHICS APPROVAL from LSHTM*

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LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

**Observational / Interventions Research Ethics Committee**

Ms Ludmila Lobkowicz
LSHTM

17 June 2019

Dear Ludmila

**Study Title:** Co-circulating Chikungunya virus, Dengue virus and Zika virus in Latin America between 2015-2017.

**LSHTM Ethics Ref:** 16412

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

**Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

**Conditions of the favourable opinion**

Approval is dependent on local ethical approval having been received, where relevant.

**Approved documents**

The final list of documents reviewed and approved by the Committee is as follows:

| Document Type | File Name | Date | Version |
|---|---|---|---|
| Protocol / Proposal | StudyProtocol_cohort pregnant women study | 01/01/2016 | 1 |
| Protocol / Proposal | WP1_Task1_Recife_0 | 01/01/2016 | 1 |
| Protocol / Proposal | WP1_Task1_Recife_1 | 01/01/2016 | 1 |
| Consent form | Consentform-cohort pregnant women study | 01/01/2016 | 1 |
| Local Approval | Recife_cohort pregnant women study_ Ethics approval | 01/01/2019 | 1 |
| Investigator CV | CV Ludmila Lobkowicz 25.1.19 | 25/01/2019 | 1 |
| Investigator CV | LSHTM_CV_Brickley_Template_February_26_2019 | 26/02/2019 | 1 |
| Protocol / Proposal | PhD study protocol | 04/04/2019 | 1 |
| Covering Letter | Cover Letter-LSHTM_Ethics_Ref_16412_4.4.19 | 04/04/2019 | 1 |
| Covering Letter | Cover Letter-LSHTM_Ethics_Ref_16412_30.5.19 | 30/05/2019 | 1 |

**After ethical review**

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

**Professor John DH Porter**
**Chair**

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

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## *APPENDIX II PAPER I SUPPLEMENTARY MATERIALS*



**SUPPLEMENTARY APPENDIX**

*Supplementary Table 1* ***Detail of cases in cross-sectional studies and case series (Table 2 & 3) reporting on signs and symptoms of qRT-PCR-confirmed ZIKV co-infections.***

| Author (year) | Location | Study year | Sex | Age (y) | Co–infecting agent | Other pathogens tested (negative) | WHO ZIKV signs or symptoms | | | | | | Other reported signs or symptoms | | Additional information |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Rash | Fever | Arthralgia | Conjunctivitis | Myalgia | Headache | URT symptoms | GI symptoms | |
| **Acevedo *et al.* (2017)** | Ecuador | 2016 | F | 54 | **CHIKV** | DENV; Gram-stain, HSV-1/2/6, CMV, EBV, VZ, Toxo, MTB, enterovirus [a] | NR | ■ | NR | NR | NR | ■ | NR | NR | Lumbar pain **Complications:** Guillain-Barré Syndrome (EMG confirmed)[c] |
| **Acevedo *et al.*(2017)** | Ecuador | 2016 | M | 44 | **CHIKV** | [a] | NR | ■ | ■ | NR | NR | NR | NR | NR | NR |
| **Acevedo *et al.*(2017)** | Ecuador | 2016 | M | 47 | **CHIKV** | [a] | NR | ■ | NR | NR | NR | NR | NR | NR | Sleep disturbance **Complications:** Tremors and walking difficulties. Tonic-clonic seizure, Encephalitis |
| **Cabral-Castro *et al.*(2016)** | Brazil | 2015/16 | F | 28 | **CHIKV** | DENV | ■ | ■ | □ | □ | NR | NR | NR | ■ | Retro-ocular pain **Complications:** Post infection relapse with pruritic rash no fever |
| **Mercado-Reyes *et al.* (2018)[b]** | Colombia | 2015/16 | NR | 47 | **CHIKV** | DENV | □ | □ | □ | NR | NR | NR | NR | NR | **Comorbidities:** Hypertension, diabetes mellitus, obesity |



Lobkowicz L, *et al*. *BMJ Global Health* 2020; 5:e002350. doi: 10.1136/bmjgh-2020-002350

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | **Complications:** Neurological syndrome<br>**Outcome:** Death |
| **Mercado-Reyes *et al.* (2018)** [b] | Colombia | 2015/16 | NR | 78 | **CHIKV** | DENV | □ | □ | □ | NR | NR | NR | NR | NR | **Comorbidities:** Hypertension, diabetes mellitus, chronic kidney disease, chronic cardiac disease<br>**Complications:** Neurological syndrome<br>**Outcome:** Death |
| **Mercado-Reyes *et al.* (2018)** [b] | Colombia | 2015/16 | NR | 28 | **CHIKV** | DENV | □ | ■ | ■ | NR | NR | NR | NR | NR | **Complications:** Haemorrhagic manifestations, Multi organ failure<br>**Outcome:** Death |
| **Mercado-Reyes *et al.* (2018)** [b] | Colombia | 2015/16 | F | 22 | **CHIKV** | DENV | NR | NR | NR | NR | NR | NR | NR | NR | Infection in pregnancy<br>**Complications** : Intrauterine growth restriction, ancephaly<br>**Outcome:** Fetal death |
| **Mercado-Reyes *et al.* (2018)** [b] | Colombia | 2015/16 | F | 22 | **CHIKV** | DENV | NR | NR | NR | NR | NR | NR | NR | NR | Infection in pregnancy<br>**Complications** : absence of heart beat<br>**Outcome:** Fetal death |
| **Metha *et al.* (2018)** | Brazil | 2015/16 | M | 34 | **CHIKV** | DENV | ■ | NR | NR | NR | NR | NR | NR | NR | **Complications:** Guillain-Barré Syndrome (EMG confirmed)[c]<br>**Outcome:** Full recovery |
| **Metha *et al.* (2018)** | Brazil | 2015/16 | M | 65 | **CHIKV** | DENV | ■ | ■ | ■ | NR | NR | NR | NR | NR | **Complications:** Myeloradiculitis<br>**Outcome:** Full recovery |
| **Sardi *et al.* (2016)** | Brazil | 2015 | F | 48 | **CHIKV** | DENV | □ | ■ | ■ | □ | ■ | ■ | NR | ■ | **Complications:** Severe arthralgia<br>**Outcome**: Sequelae |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sardi *et al.* (2016)** | Brazil | 2015 | F | 40 | **CHIKV** | DENV | ■ | ■ | NR | ■ | ■ | NR | NR | NR | Painful posterior cervical lymph node measuring 5 mm **Outcome:** Full recovery |
| **Alva-Urcia *et al.*(2016)** | Peru | 2016 | NR | NR | **DENV** | NR | NR | ■ | NR | NR | NR | NR | NR | NR | NR |
| **Silva *et al.* (2019)** | Brazil | 2014/16 | NR | NR | **DENV** | CHIKV | ■ | NR | ■ | NR | NR | ■ | NR | ■ | Retro-orbital pain |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | F | 63 | **DENV 1** | CHIKV | ■ | ■ | ■ | NR | ■ | ■ | NR | NR | NR |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | M | 27 | **DENV 1** | CHIKV | ■ | ■ | NR | NR | ■ | NR | NR | NR | NR |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | F | 64 | **DENV 1** | CHIKV | NR | NR | NR | NR | NR | NR | NR | ■ | NR |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | M | 68 | **DENV 1** | CHIKV | NR | ■ | ■ | NR | ■ | ■ | NR | NR | **Complications** : Abrupt platelets decrease |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | M | 33 | **DENV 1** | CHIKV | NR | ■ | NR | NR | ■ | ■ | NR | ■ | **Complications** : Abrupt platelets decrease |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | M | 62 | **DENV 1** | CHIKV | NR | ■ | NR | NR | ■ | ■ | NR | NR | NR |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | F | 36 | **DENV 1** | CHIKV | ■ | ■ | NR | NR | ■ | ■ | NR | NR | NR |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | M | 25 | **DENV 2** | CHIKV | ■ | ■ | NR | ■ | ■ | ■ | NR | ■ | NR |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | F | 39 | **DENV 2** | CHIKV | ■ | ■ | ■ | NR | NR | NR | NR | NR | NR |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | M | 48 | **DENV 2** | CHIKV | ■ | ■ | ■ | ■ | ■ | ■ | NR | NR | NR |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Estofolete *et al.*(2018)** | Brazil | 2016 | F | 47 | **DENV 2** | CHIKV | ■ | NR | ■ | ■ | ■ | ■ | NR | ■ | NR |
| **Estofolete *et al.*(2018)** | Brazil | 2016 | M | 41 | **DENV 2** | CHIKV | NR | NR | ■ | NR | ■ | ■ | NR | ■ | NR |
| **Chia *et al.* (2017)** | Singa-pore | 2016 | M | 42 | **DENV 3** | NR | ■ | ■ | NR | ■ | ■ | ■ | NR | ■ | NR |
| **Chia *et al.* (2017)** | Singa-pore | 2016 | F | 45 | **DENV 1** | NR | ■ | ■ | NR | NR | ■ | NR | NR | ■ | **Complications :** Malaise, gingival bleeding |
| **Chia *et al.* (2017)** | Singa-pore | 2016 | M | 40 | **DENV 1** | NR | ■ | ■ | ■ | ■ | ■ | NR | NR | ■ | NR |
| **Chia *et al.* (2017)** | Singa-pore | 2016 | M | 15 | **DENV** | NR | ■ | ■ | ■ | NR | NR | ■ | ■ | NR | **Complications :** Developed significant thrombocytopenia |
| **Li *et al.* (2017)** | Sing-apore | 2016 | M | 15 | **DENV** | CHIKV | ■ | ■ | ■ | ■ | ■ | ■ | □ | NR | Bilateral knee pain<br>**Outcome:** Full recovery |
| **Acevedo *et al.*(2017)** | Ecuador | 2016 | M | 18 | **CHIKV, DENV** | [a] | NR | NR | NR | NR | NR | NR | ■ | NR | **Complications:** Guillain-Barré Syndrome (EMG confirmed)[c] |
| **Acevedo *et al.*(2017)** | Ecuador | 2016 | M | 23 | **CHIKV, DENV** | [a] | NR | NR | NR | NR | NR | NR | NR | NR | **Complications:** Encephalitis<br>**Outcome:** Death |
| **Acevedo *et al.*(2017)** | Ecuador | 2016 | F | 25 | **CHIKV, DENV** | [a] | NR | ■ | NR | NR | NR | ■ | NR | ■ | Followed by generalized tonic-clonic seizure, ptosis of the eyelids, neck stiffness<br>**Complications:** Meningitis |
| **Acevedo *et al.*(2017)** | Ecuador | 2016 | M | 62 | **CHIKV, DENV** | [a] | NR | ■ | NR | NR | NR | NR | NR | NR | Sweating, paraplegia, arreflexia, dyspnea, decreased muscular strength in arms<br>**Complications :** Guillain-Barré Syndrome (EMG confirmed)[c] |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Acevedo et al.(2017)** | Ecuador | 2016 | F | 28 | **CHIKV HIV, Toxo** | [a] | NR | ■ | NR | NR | NR | ■ | NR | NR | **Complications:** Meningitis |
| **Souza Costa et al. (2019)** | Brazil | 2015/16 | F | 35 | **MAYV** | DENV, YFV, SLEV, ILHV, ROCV, WNV, EEEV,WEEV, VEEV | NR | NR | NR | NR | NR | NR | NR | NR | Infection in third trimester of pregnancy **Outcome:** Newborn presented without any congenital abnormalities |

■ *Reported to be present;* □ *Reported not to be present;* *Not reported (**NR**); Upper Respiratory Tract (**URT**) symptoms: pharyngitis, sore throat, cough, pharyngeal congestion, adenopathy; Gastro-intestinal (**GI**) symptoms: nausea, diarrhea, vomiting, constipation, stomach ache; Zika virus (**ZIKV**); Chikungunya virus (**CHIKV**); Dengue virus (**DENV**); Herpes simplex virus (**HSV**); Cytomegalovirus (**CMV**); Varizella zoster (**VZ**); Toxoplasma gondii ( **Toxo**); Mycobacterium tuberculosis (**MTB**); Mayaro virus (**MAYV**); Yellow fever virus (**YFV**); West Nile virus (**WNV**); Saint Louis Encephalitis virus (**SLEV**); Rocio virus (**ROCV**); Ilheus virus (**ILHV**); East, West, Venezuelan equine encephalitis virus (**EEV,WEEV,VEEV**), Electromyography testing ( **EMG**);* [a] *tested for* DENV; Gram-stain; HSV-1/2/6; CMV; EBV; VZ; Toxo; MTB; enterovirus. [b] *Details were only reported on five fatal ZIKV/CHIKV cases.* [c] *Electromyography testing ( **EMG**).*

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*Supplementary Table 2* ***Details of study populations in cohort studies, cross-sectional studies, and case series (n = 20 studies).***

| Author (year) | Study population | Details of study population as described in publication |
|---|---|---|
| **Acevedo et *al.* (2017)** | Cases with neurological symptoms and suspected arbovirus infections | Cases with neurological symptoms and/ or concern for acute arboviral infections without case definition. |
| **Alva-Urcia et *al.* (2016)** | AFI cases | Cases who arrived to Internal Medicine-Pediatrics outpatient clinics with AFI (greater than or equal to 38˚C axillary temperature in the previous 7 days) along with one or more of the following symptoms: headache, muscle pain, retro-ocular pain, joint pain, nausea, low appetite, vomiting, dizziness, abdominal pain, diarrhea, chills, rash, photophobia, sore throat, cough, pallor, rhinorrhea, dyspnea, jaundice, cough, conjunctival injection, dysuria or convulsions; no cases with an identifiable source of infection, such as sinusitis, pneumonia, acute otitis media and acute upper respiratory tract infections, among others. |
| **Azeredo et *al.* (2018)** | Suspected arbovirus infections | Cases with fever and rash during acute phase of infection (up to the 7th day after disease onset) followed by at least two of the following signals and symptoms: headache, myalgia or arthralgia, conjunctivitis, pruritus, retro-orbital pain and prostration were recruited as suspicion of arboviral infection. |
| **Ball et *al.* (2018)** | AFI cases | Cohort of school children of acute undifferentiated febrile illness (AFI).AFI defined as cases with fever and/or fever on presentation in a child with no obvious source of infection (ie, no respiratory symptoms, symptoms of urinary tract infection, or diagnostic criteria for malaria or typhoid). |
| **Brasil et *al.* (2016)** | Pregnant women with rash | Pregnant women with a rash that had developed in the previous 5 days. |
| **Cabral-Castro et *al.* (2016)** | Suspected DENV infections | Suspected DENV cases without case definition. |
| **Carrillo-Hernandez et *al.* (2018)** | Suspected arbovirus infections | Cases with febrile syndrome compatible with ZIKV, CHIKV and DENV infection, and in the acute phase of the disease, i.e., fever for no more than seven days. |
| **Charlys da Costa et *al.* (2017)** | Suspected arbovirus infections with rash | Cases with exanthemous illness symptoms compatible with ZIKV, CHIKV and DENV infection. |
| **Chia et *al.* (2017)** | Suspected ZIKV infections | Suspected ZIKV cases with fever, maculopapular rash, and any of the following: arthralgia, myalgia, headache, or non-purulent conjunctivitis. |

| | | |
|---|---|---|
| **Souza Costa *et al.* (2019)** | AFI cases | Cases notified as suspected ZIKV, CHIKV and DENV cases with National Surveillance System of Public Health (SINAN) of Brazil. |
| **Colombo *et al.* (2017)** | Suspected ZIKV infections | Cases with macular or papular rash and two or more of the following signs and symptoms: fever or conjunctival hyperemia without secretion and pruritus, polyarthralgia or joint edema |
| **Estofolete *et al.* (2018)** | Suspected arbovirus infections | Suspected DENV cases: fever, abdominal pain, vomiting, bleeding of the mouth, hemorrhage, volume of urine, breathing difficulties, feeling cold, and suspected Zika cases presence of macular or papular rash with two or more of following signs and symptoms: fever or conjunctival hyperemia without secretion. |
| **Li *et al.* (2017)** | Suspected ZIKV infections | Suspected ZIKV cases with fever, maculopapular rash, and any of the following: arthralgia, myalgia, headache, and non-purulent conjunctivitis. |
| **Magalhaes *et al.* (2017)** | Suspected arbovirus infections | Cases had to be older the age of 5 years and have fever or history of fever for less than 72 h, clinical symptoms were supposed to be consistent with possible dengue, i.e., suspicion of dengue and/or undifferentiated fever in a patient from a dengue endemic area however with no signs of severe disease. Cases were not included if there were localized features suggesting an alternative diagnosis, e.g., pneumonia, otitis, etc. |
| **Mercado-Reyes *et al.* (2018)** | Suspected arbovirus infections | Cases with suspected ZIKV, CHIKV and DENV infections, which was reported to the National Surveillance System of Public Health (SIVIGILA) of Colombia. |
| **Metha *et al.* (2018)** | Cases with neurological symptoms and suspected ZIKV infections | Cases with an acute neurological condition associated with a suspected ZIKV infection, as identified by fever, arthralgia or rash illness in the preceding three months. |
| **Pessoa *et al.* (2016)** | Suspected arbovirus infections | Cases with rash, conjunctivitis, and/or joint pain with or without fever or headache were examined further. |
| **Sardi *et al.* (2016)** | AVI and of qRT-PCR ZIKV+ infections | Acute viral illness without case definition, and positive ZIKV qRT-PCR result. |
| **Silva *et al.* (2019)** | Suspected arbovirus infections | Cases with suspected ZIKV, CHIKV and DENV infections . |
| **Waggoner *et al.* (2016)** | Suspected arbovirus infections | Cases with suspected ZIKV, CHIKV, and/or DENV infections. |

*Acute Febrile Illness (**AFI**); Acute Viral illness (**AVI**); Zika virus (**ZIKV**); Chikungunya virus (**CHIKV**); Dengue virus (**DENV**);*

*Supplementary Appendix 1.* ***Literature Search Strategy: Search terms for each database***

**Pubmed:**

(zikv OR zika OR "Zika Virus"[Mesh] OR "Zika Virus Infection"[Mesh]) AND (coinfec* OR (co infec*) OR co-infec* OR "Coinfection"[Mesh] OR "Bacterial Infections"[Mesh] OR bacterial* OR bacteria* OR bacteri* OR bactéri* OR "Parasitic Diseases"[Mesh] OR "parasite infections" OR parsit* OR parasitic OR parasitical OR parásit* OR TORCH OR "Toxoplasmosis"[Mesh] OR toxoplasm* OR "Syphilis"[Mesh] OR sífilis OR syphilis OR "Rubella"[Mesh] OR rubéol* OR rubel* OR "Cytomegalovirus"[Mesh] OR citomegalovirus OR cytomegalovirus OR cytomégalovirus OR "Herpes Simplex"[Mesh] OR herpes OR herp* OR "Simplexvirus"[Mesh] OR "Trematode Infections"[Mesh] OR tremat* OR trématode* OR "Filariasis"[Mesh] OR filari* OR "Onchocerciasis"[Mesh] OR Onchocerciasis OR Oncocercos* OR "Schistosoma"[Mesh] OR schisto* OR "Helminths"[Mesh] OR helmin* OR "Rabies"[Mesh] OR rabi* OR raiva OR rage OR "Trachoma"[Mesh] OR trachom* OR tracoma OR "Yaws"[Mesh] OR yaws OR bouba OR pian OR "Leprosy"[Mesh] OR leprosy OR lepra OR lèpre OR "Chagas disease"[Mesh] OR chagas OR "Leishmaniasis"[Mesh] OR leishmani* OR "Taeniasis"[Mesh] OR Taenia* OR "Echinococcus"[Mesh] OR echinoc* OR échinoc* OR equinococo OR "Neurocysticercosis"[Mesh] OR "Neglected Diseases"[Mesh] OR "neglected tropical" OR denv OR dengue OR "Dengue Virus"[Mesh] OR chikv OR chikungunya OR "Chikungunya Virus"[Mesh] OR wnv OR "west nile virus" OR "West Nile Virus"[Mesh] OR yfv OR "Yellow fever virus" OR "Yellow fever Virus"[Mesh] OR "Encephalitis Virus, Japanese"[Mesh] OR "Japanese Encephalitis Virus" OR jev OR "Encephalitis virus, St. Louis "[Mesh] OR "St. Louis encephalitis virus" OR slev OR "Kunjin virus" OR "Murray Valley encephalitis virus" OR mvev OR "Usutu virus"[Mesh] OR "Usutu virus" OR usuv OR "Encephalitis Viruses, Tick-Borne"[Mesh] OR "Tick-Borne Encephalitis Viruses" OR tbev OR "Rift Valley fever virus"[Mesh] OR "Rift Valley fever virus" OR rvfv OR OR hiv OR "human immunodeficiency virus" OR "virus da imunodeficiencia adquirida" OR "virus da imunodeficiencia humana" OR "virus da inmunodeficiencia adquirida" OR "virus da inmunodeficiencia humana" OR "HIV"[Mesh] OR AIDS OR "Respiratory Syncytial Viruses"[Mesh] OR "respiratory viral infcetion" OR RSV OR "influenza,human"[Mesh] OR influenza OR "Adenovirus Infections, Human"[Mesh] OR Adenov* OR adénovirus OR "Enterovirus"[Mesh] OR enterov* OR entérovirus OR "Tuberculosis"[Mesh] OR Tuberculos* OR astrovirus OR "Norovirus"[Mesh] OR norovirus OR "Rotavirus Infections"[Mesh] OR rotavirus OR "Giardiasis"[Mesh] OR giardias* OR giard* OR "Amebiasis" OR ameb* OR amoebiasis OR amibiase)

**Web of Science:**

(zikv OR zika OR "Zika Virus"[Mesh] OR "Zika Virus Infection"[Mesh]) AND (coinfec* OR (co infec*) OR co-infec* OR Coinfection[Mesh] OR "Bacterial Infections"[Mesh] OR bacterial* OR bacteria* OR bacteri* OR bactéri* OR "Parasitic Diseases"[Mesh] OR "parasite infections" OR parsit* OR parasitic OR parasitical OR parásit* OR TORCH OR "Toxoplasmosis"[Mesh] OR

toxoplasm* OR "Syphilis"[Mesh] OR sifilis OR syphilis OR Rubella[Mesh] OR rubéol* OR rubel* OR Cytomegalovirus[Mesh] OR citomegalovirus OR cytomegalovirus OR cytomégalovirus OR "Herpes Simplex"[Mesh] OR herpes OR herp* OR Simplexvirus[Mesh] OR "Trematode Infections"[Mesh] OR tremat* OR trématode* OR Filariasis[Mesh] OR Filari* OR Onchocerciasis[Mesh] OR Onchocerciasis OR Oncocercos* OR Schistosoma[Mesh] OR schisto* OR Helminths[Mesh] OR helmin* OR Rabies[Mesh] OR rabi* OR raiva OR rage OR Trachoma[Mesh] OR trachom* OR tracoma OR Yaws[Mesh] OR yaws OR bouba OR pian OR Leprosy[Mesh] OR leprosy OR lepra OR lèpre OR "Chagas disease"[Mesh] OR chagas OR Leishmaniasis[Mesh] OR leishmani* OR Taeniasis[Mesh] OR Taenia* OR Echinococcus[Mesh] OR echinoc* OR échinoc* OR equinococo OR Neurocysticercosis[Mesh] OR Neglected Diseases[Mesh] OR "neglected tropical" OR denv OR dengue OR "Dengue Virus"[Mesh] OR chikv OR chikungunya OR "Chikungunya Virus"[Mesh] OR wnv OR "west nile virus" OR "West Nile Virus"[Mesh] OR yfv OR "Yellow fever virus" OR "Yellow fever Virus"[Mesh] OR "Encephalitis Virus, Japanese"[Mesh] OR "Japanese Encephalitis Virus" OR jev OR "Encephalitis virus, St. Louis "[Mesh] OR "St. Louis encephalitis virus" OR slev OR "Kunjin virus" OR "Murray Valley encephalitis virus" OR mvev OR "Usutu virus"[Mesh] OR "Usutu virus" OR usuv OR "Encephalitis Viruses, Tick-Borne"[Mesh] OR "Tick-Borne Encephalitis Viruses" OR tbev OR "Rift Valley fever virus"[Mesh] OR "Rift Valley fever virus" OR rvfv OR hiv OR "human immunodeficiency virus" OR "virus da imunodeficiencia adquirida" OR "virus da imunodeficiencia humana" OR "virus da inmunodeficiencia adquirida" OR "virus da inmunodeficiencia humana" OR HIV[Mesh] OR AIDS OR "Respiratory Syncytial Viruses"[Mesh] OR "respiratory viral infection" OR RSV OR "influenza,human"[Mesh] OR influenza OR "Adenovirus Infections, Human"[Mesh] OR Adenov* OR adénovirus OR Enterovirus[Mesh] OR enterov* OR entérovirus OR Tuberculosis[Mesh] OR Tuberculos* OR astrovirus OR Norovirus[Mesh] OR norovirus OR "Rotavirus Infections"[Mesh] OR rotavirus OR Giardiasis[Mesh] OR giardias* OR giard* OR Amebiasis OR ameb* OR amoebiasis OR amibiase)

**Embase:**

(zikv OR zika ) AND (coinfec* OR "co infection" OR "co infections" OR (co-infec*) OR bacterial* OR bacteria* OR bacteri* OR bacteri* OR "parasite infections" OR parsit* OR parasitic OR parasitical OR parasit* OR TORCH OR toxoplasm* OR sifilis OR syphilis OR rubeol* OR rubel* OR citomegalovirus OR cytomegalovirus OR cytomegalovirus OR herpes OR herp* OR tremat* OR trematode* OR Filari* OR Onchocerciasis OR Oncocercos* OR schisto*OR helmin* OR rabi* OR raiva OR rage OR trachom* OR tracoma OR yaws OR bouba OR pian OR leprosy OR lepra OR lepre OR chagas OR leishmani* OR Taenia* OR echinoc* OR echinoc* OR equinococo OR "neglected tropical" OR denv OR dengue OR chikv OR chikungunya OR wnv OR "west nile virus" OR yfv OR "Yellow fever virus" OR "Japanese Encephalitis Virus" OR jev OR "St. Louis encephalitis virus" OR slev OR "Kunjin virus" OR "Murray Valley encephalitis virus" OR mvev OR "Usutu virus" OR usuv OR "Tick-Borne Encephalitis Viruses" OR tbev OR "Rift Valley fever virus" OR rvfv OR hiv OR "human immunodeficiency virus" OR "virus da imunodeficiencia adquirida" OR "virus da imunodeficiencia humana" OR "virus da inmunodeficiencia adquirida" OR "virus da inmunodeficiencia humana" OR AIDS OR "respiratory viral infection"

OR RSV OR influenza OR Adenov* OR adenovirus OR enterov* OR enterovirus OR Tuberculos* OR astrovirus OR norovirus OR rotavirus OR giardias* OR giard* OR "Amebiasis" OR ameb* OR amoebiasis OR amibiase)

**LILACs:**

((tw:(zikv)) OR (tw:(zika))) AND ((tw:(coinfec*)) OR (tw:(co-infec*)) OR (tw:( bacterial*)) OR (tw:( bacteria*)) OR (tw:( bacteri*)) OR (tw:("parasite infections" )) OR (tw:(parasit* )) OR (tw:(parasitic)) OR (tw:( parasitical )) OR (tw:(parásit* )) OR (tw:(TORCH)) OR (tw:(toxoplasm*)) OR (tw:(sífilis)) OR (tw:(syphilis)) OR (tw:(rubéol* )) OR (tw:(rubel* )) OR (tw:(citomegalovirus)) OR (tw:(cytomegalovirus )) OR (tw:(cytomégalovirus)) OR (tw:(herpes )) OR (tw:(herp* )) OR (tw:(tremat* )) OR (tw:(trématode* )) OR (tw:(Filari* )) OR (tw:(Onchocerciasis)) OR (tw:( Oncocercos* )) OR (tw:(schisto*)) OR (tw:(helmin*)) OR (tw:(rabi*)) OR (tw:(raiva )) OR (tw:(rage )) OR (tw:(trachom* )) OR (tw:(tracoma)) OR (tw:(yaws )) OR (tw:(bouba)) OR (tw:(pian )) OR (tw:( leprosy)) OR (tw:(lepra)) OR (tw:( lèpre)) OR (tw:(chagas )) OR (tw:(leishmani* )) OR (tw:( Taenia*)) OR (tw:(echinoc*)) OR (tw:(échinoc)) OR (tw:(equinococo )) OR (tw:( "neglected tropical" )) OR (tw:( denv )) OR (tw:(dengue)) OR (tw:(chikv)) OR (tw:(chikungunya )) OR (tw:(wnv)) OR (tw:( "west nile virus" )) OR (tw:(yfv)) OR (tw:( "Yellow fever virus")) OR (tw:("Japanese Encephalitis Virus")) OR (tw:(jev)) OR (tw:( "St. Louis encephalitis virus")) OR (tw:( slev)) OR (tw:( "Kunjin virus" )) OR (tw:("Murray Valley encephalitis virus" )) OR (tw:(mvev)) OR (tw:("Usutu virus" )) OR (tw:(usuv)) OR (tw:("Tick-Borne Encephalitis Viruses" )) OR (tw:(tbev)) OR (tw:( "Rift Valley fever virus")) OR (tw:(rvfv)) OR (tw:(hiv)) OR (tw:(aids)) OR (tw:("human immunodeficiency virus" )) OR (tw:("virus da imunodeficiencia adquirida" )) OR (tw:("virus da imunodeficiencia humana" )) OR (tw:("virus da inmunodeficiencia adquirida" )) OR (tw:( "virus da inmunodeficiencia humana" )) OR (tw:("respiratory viral infection" )) OR (tw:(rsv)) OR (tw:(influenza)) OR (tw:(Adenov* )) OR (tw:(adénovirus )) OR (tw:(enterov*)) OR (tw:(entérovirus )) OR (tw:(Tuberculos* )) OR (tw:( astrovirus )) OR (tw:(norovirus )) OR (tw:(rotavirus)) OR (tw:(giardias* )) OR (tw:(giard* )) OR (tw:("Amebiasis" )) OR (tw:(ameb* )) OR (tw:(amoebiasis )) OR (tw:(amibiase)))

*Supplementary Appendix 2.* ***Oxford Centre for Evidence-based Medicine – Level of Evidence. Table replicated from: https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/*** [11].

| Level | Therapy / Prevention, Aetiology / Harm | Prognosis | Diagnosis | Differential diagnosis / symptom prevalence study | Economic and decision analyses |
|---|---|---|---|---|---|
| **1a** | SR (with homogeneity*) of RCTs | SR (with homogeneity*) of inception cohort studies; CDR" validated in different populations | SR (with homogeneity*) of Level 1 diagnostic studies; CDR" with 1b studies from different clinical centres | SR (with homogeneity*) of prospective cohort studies | SR (with homogeneity*) of Level 1 economic studies |
| **1b** | Individual RCT (with narrow Confidence Interval";) | Individual inception cohort study with > 80% follow-up; CDR" validated in a single population | Validating** cohort study with good" " " reference standards; or CDR" tested within one clinical centre | Prospective cohort study with good follow-up**** | Analysis based on clinically sensible costs or alternatives; systematic review(s) of the evidence; and including multi-way sensitivity analyses |
| **1c** | All or none§ | All or none case series | Absolute SpPins and SnNouts" " | All or none case series | Absolute better-value or worse-value analyses " " " " |
| **2a** | SR (with homogeneity*) of cohort studies | SR (with homogeneity*) of either retrospective cohort studies or untreated control groups in RCTs | SR (with homogeneity*) of Level >2 diagnostic studies | SR (with homogeneity*) of 2b and better studies | SR (with homogeneity*) of Level >2 economic studies |
| **2b** | Individual cohort study (including low quality RCT; e.g., <80% follow-up) | Retrospective cohort study or follow-up of untreated control patients in an RCT; Derivation of CDR" or validated on split-sample§§§ only | Exploratory** cohort study with good" " " reference standards; CDR" after derivation, or validated only on split-sample§§§ or databases | Retrospective cohort study, or poor follow-up | Analysis based on clinically sensible costs or alternatives; limited review(s) of the evidence, or single studies; and including multi-way sensitivity analyses |
| **2c** | "Outcomes" Research; Ecological studies | "Outcomes" Research | | Ecological studies | Audit or outcomes research |

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| | | | | | |
|---|---|---|---|---|---|
| **3a** | SR (with homogeneity*) of case-control studies | | SR (with homogeneity*) of 3b and better studies | SR (with homogeneity*) of 3b and better studies | SR (with homogeneity*) of 3b and better studies |
| **3b** | Individual Case-Control Study | | Non-consecutive study; or without consistently applied reference standards | Non-consecutive cohort study, or very limited population | Analysis based on limited alternatives or costs, poor quality estimates of data, but including sensitivity analyses incorporating clinically sensible variations. |
| **4** | Case series (and poor quality cohort and case-control studies§§) | Case series (and poor quality prognostic cohort studies***) | Case-control study, poor or non-independent reference standard | Case series or superseded reference standards | Analysis with no sensitivity analysis |
| **5** | Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles" | Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles" | Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles" | Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles" | Expert opinion without explicit critical appraisal, or based on economic theory or "first principles" |

* By homogeneity we mean a systematic review that is free of worrisome variations (heterogeneity) in the directions and degrees of results between individual studies. Not all systematic reviews with statistically significant heterogeneity need be worrisome, and not all worrisome heterogeneity need be statistically significant. As noted above, studies displaying worrisome heterogeneity should be tagged with a "-" at the end of their designated level.

" Clinical Decision Rule. (These are algorithms or scoring systems that lead to a prognostic estimation or a diagnostic category.)

"¡ See note above for advice on how to understand, rate and use trials or other studies with wide confidence intervals.

§ Met when all patients died before the Rx became available, but some now survive on it; or when some patients died before the Rx became available, but none now die on it.

§§ By poor quality cohort study we mean one that failed to clearly define comparison groups and/or failed to measure exposures and outcomes in the same (preferably blinded), objective way in both exposed and non-exposed individuals and/or failed to identify or appropriately control known confounders and/or failed to carry out a sufficiently long and complete follow-up of patients. By poor quality case-control study we mean one that failed to clearly define comparison

groups and/or failed to measure exposures and outcomes in the same (preferably blinded), objective way in both cases and controls and/or failed to identify or appropriately control known confounders.

§§§ Split-sample validation is achieved by collecting all the information in a single tranche, then artificially dividing this into "derivation" and "validation" samples.

" " An "Absolute SpPin" is a diagnostic finding whose Specificity is so high that a Positive result rules-in the diagnosis. An "Absolute SnNout" is a diagnostic finding whose Sensitivity is so high that a Negative result rules-out the diagnosis.

";¡"¡ Good, better, bad and worse refer to the comparisons between treatments in terms of their clinical risks and benefits.

" " " Good reference standards are independent of the test, and applied blindly or objectively to applied to all patients. Poor reference standards are haphazardly applied, but still independent of the test. Use of a non-independent reference standard (where the 'test' is included in the 'reference', or where the 'testing' affects the 'reference') implies a level 4 study.

" " " " Better-value treatments are clearly as good but cheaper, or better at the same or reduced cost. Worse-value treatments are as good and more expensive, or worse and the equally or more expensive.

** Validating studies test the quality of a specific diagnostic test, based on prior evidence. An exploratory study collects information and trawls the data (e.g. using a regression analysis) to find which factors are 'significant'.

*** By poor quality prognostic cohort study we mean one in which sampling was biased in favour of patients who already had the target outcome, or the measurement of outcomes was accomplished in <80% of study patients, or outcomes were determined in an unblinded, non-objective way, or there was no correction for confounding factors.

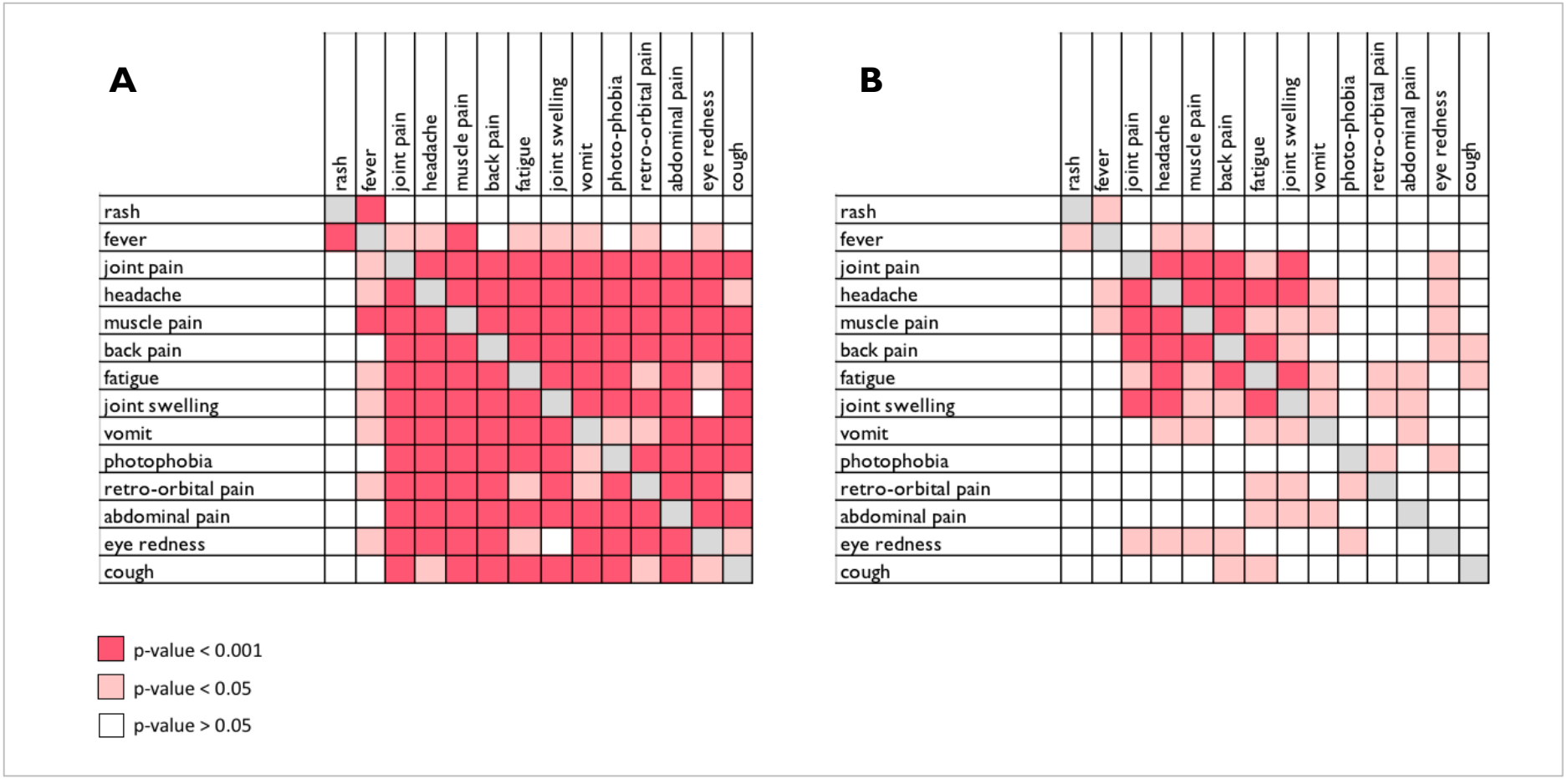
**** Good follow-up in a differential diagnosis study is >80%, with adequate time for alternative diagnoses to emerge (for example 1-6 months acute, 1 - 5 years chronic)

## *APPENDIX II PAPER II SUPPLEMENTARY MATERIALS*

*Supplementary table 1:* Odds ratio of being CHIKV mono-infected compared to ZIKV mono- infected, of significantly common symptoms of CHIKV mono-infected pregnant women were combined in a model.

| Symptom | Variable | OR of being CHIKV infected vs. ZIKV infected | 95 % CI | p-value* |
|---|---|---|---|---|
| Joint pain | joint pain | 3.01 | (1.64- 5.52) | 0.0001 |
| Joint pain + joint swelling | joint pain | 2.3 | (1.13- 4.73) | 0.022 |
| | joint swelling | 1.78 | (0.81- 3.94) | 0.153 |
| Joint pain + joint swelling+ fatigue | joint pain | 2.13 | (1.00- 4.52) | 0.49 |
| | joint swelling | 1.63 | (0.71- 3.75) | 0.246 |
| | fatigue | 1.33 | (0.60- 2.95) | 0.54 |
| Joint pain + fatigue | joint pain | 2.52 | (1.26- 5.02) | 0.009 |
| | fatigue | 1.53 | (0.71- 3.25) | 0.273 |
| Joint pain + joint swelling + fatigue +headache | joint pain | 2.18 | (0.94- 5.05) | 0.07 |
| | joint swelling | 1.63 | (0.71- 3.78) | 0.244 |
| | fatigue | 1.33 | (0.59- 3.07) | 0.476 |
| | headache | 0.15 | (0.41- 2.23) | 0.913 |
| Joint pain + headache | joint pain | 2.76 | (1.27- 6.00) | 0.01 |
| | headache | 1.15 | (0.52- 2.54) | 0.731 |
| Fatigue + headache | fatigue | 1.89 | (0.87- 4.12) | 0.107 |
| | headache | 1.64 | (0.79- 3.40) | 0.181 |
| Fatigue +joint pain+ headache | fatigue | 1.52 | (0.69- 3.37) | 0.299 |
| | joint pain | 2.51 | (1.13- 5.58) | 0.024 |
| | headache | 1.00 | (0.44- 2.32) | 0.988 |

*p-values from likelihood ratio test




*Supplementary Figure 1:* **Presence of one symptom being associated with the presence of another symptom.** In **A** association of symptom presentation is presented for only ZIKV cases and in **B** association of symptom presentation is presented only for CHIKV cases. Association was tested using a chi-squared test. Dark red depicted a p-value of <0.001, pink depicted a p-value of <0.05 and white depicted no significant p-value.